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NOTES ON TYPE SPECIMENS OF BRITISH INOPERCULATE DISCOMYCETES

(FIRST PART, NOTES 1–50)

By J. A. NANNFELDT
(*Botaniska Institutionen, Upsala*)

A grant from the University of Upsala (C. F. Liljewalch's resestipendium) made it possible for me to visit London in the summer of 1932 in order to study Discomycetes in the herbaria of the British Museum (Natural History) and of the Royal Botanic Gardens, Kew. When comparing a British fungus flora with one from the Continent one is struck by the number of species described from Britain and not known outside it. After studying the types of those British species I was able to establish the fact that most of them have been known for a long time on the Continent also, though under different names.

My main interest was devoted to the inoperculates, and in these notes the results of my examination of fifty British species of that group are given. The preparation of the list has been much delayed for various reasons, but I hope to be able to continue it soon. My work in London was greatly facilitated by the courtesy shown to me by the officials, and I wish to express my profound gratitude to them all.

In this paper the species are arranged alphabetically, according to the names in Ramsbottom's List of British Discomycetes (*Trans. Brit. mycol. Soc.* iv). For the convenience of the reader, the synonymy and citations to the British fungus floras as well as to Saccardo's *Sylloge Fungorum*, Rehm's *Discomyceten*, and Boudier's *Discomycètes d'Europe* are appended. For further information references are frequently given to v. Höhnel's numerous papers (his "Fragmente zur Mycologie" being cited as "Fr. z. M." and his "Mycologische Fragmente" as "M. Fr.") and to my own work: "Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten" in *Nova Acta Reg. Soc. Sci. upsal.* Ser. iv, vol. viii, No. 2 (cited as Nannfeldt, 1932).

1. ***Belonidium Jerdoni*** (Cke. & Phill.) Massee, *Brit. F. Fl.* iv, 229.
Peziza Jerdoni Cke. & Phill. in sched.

This name, as well as *Hyalinia incarnata* (Cke.) Boud. (note 15), is a synonym for *Pseudohelotium pineti* (Batsch ex Fr.) Fuck. This gathering was listed by Phillips as *Mollisia lurida* (Pers.) Phill. and declared to

be quite distinct from *Peziza pineti* Batsch. Phillips based his identification on a Fries specimen at Kew, labelled *P. lurida*. Massee took up a herbarium name by Cooke & Phillips for the fungus in question, stating that it is different from *P. pineti* Batsch = *P. lurida* Pers. I have studied the type of *P. Jerdoni* as well as the Fries specimen. They are both typical *Pseudohelotium pineti* as understood by v. Höhnel (Fr. z. M. No. 1224) who points out the very large variability in the shape and size of the spores of this species.

No specimen of *Peziza lurida* is to be found in Herb. Persoon (at Leyden), but the description leaves no doubt that he had the same fungus in mind.

2. ***Belonium Arctii*** (Phill.) Sacc. *Syll. F.* VIII, 495 (No. 2044); Boud. *Discom. d'Eur.* p. 118.

   Peziza (*Mollisia*) *Arctii* Phill. ap. Buckn. in *Proc. Bristol Nat. Soc.* N.S. IV, 58.

   Mollisia Arctii Phill. *Brit. Discom.* p. 183.

   Belonidium Arctii Massee, *Brit. F. Fl.* IV, 225.

   Pyrenopeziza Arctii Nannf. in *Nova Acta Reg. Soc. Sci. upsal.* Ser. IV, vol. VIII, No. 2, p. 142.

The type specimen, as well as the specimens distributed in Vize, *Micro. f. brit.* No. 476, are identical with the fungus I recently published as *Pyrenopeziza Arctii* (Phill.) Nannf. Until then it was recorded only from the type locality, but I have found it to be fairly common in Sweden.

3. ***Belonium filisporum*** (Cke.) Sacc. *Syll. F.* VIII, 494 (No. 2039); Boud. *Discom. d'Eur.* p. 118.

   Peziza (*Mollisia*) *filispora* Cke. in *Grev.* III, 66.

   Belonidium filisporum Phill. *Brit. Discom.* p. 152; Massee, *Brit. F. Fl.* IV, 226.

   Trichobelonium filisporum Rehm in *Ber. bayer. bot. Ges.* XIV, 107.

This species is said in the original description to grow "on sheaths of grass". The type specimen (in Herb. Kew.) shows that the grass is *Brachypodium sylvaticum* and the fungus is identical with *Belonopsis pallens* (Sacc.) Keissl. (For description and synonymy of this species see Nannfeldt, 1932, p. 104.) As Cooke's specific epithet antedates that of Saccardo, the fungus should be called *Belonopsis filispora* (Cke.) Nannf. n.comb.

The same species is preserved in Phillips's herbarium (Brit. Mus.) as "*Belonidium albido-virella* n.s. ined."

4. ***Calloria coniicola*** Cke. & Phill. ap. Phill. *Brit. Discom.* p. 333; Sacc. *Syll. F.* VIII, 639 (No. 2637); Massee, *Brit. F. Fl.* IV, 152; Boud. *Discom. d'Eur.* p. 101.

Judging from descriptions only, I have suggested that this species might belong to the genus *Laetinaevia* Nannf. (Nannfeldt, 1932, p. 191). The examination of the type specimen showed that the descriptions are very insufficient and that my suggestion was wrong. It is a very delicate *Lachnum*, which—so far as I understand—cannot be specifically separated from *L. brevipilum* v. Höhn.

5. ***Calloria cornea*** (B. & Br.) Phill. *Brit. Discom.* p. 332; Sacc. *Syll. F.* VIII, 640 (No. 2639); Massee, *Brit. F. Fl.* IV, 152; Boud. *Discom. d'Eur.* p. 101.

Peziza cornea B. & Br. in *Ann. Mag. nat. Hist.* (2), VII, 183 (No. 578); Berk. *Outl.* p. 371; Cke. *Handb.* p. 704 (No. 2113).

This species has been studied by v. Höhnel (e.g. Fr. z. M. No. 1074), who considered it to be a *Mollisia*, *M. cornea* (B. & Br.) v. Höhn. I supported this view (Nannfeldt, 1932, p. 126), and gave drawings of the excipulum. v. Höhnel's opinion—as well as mine—was based solely on the study of specimens distributed in Rabenhorst's *Fungi europaei*. The type specimen is identical with these.

6. ***Calloria fusarioides*** (Berk.) Fr. *S. Veg. Scand.* p. 359; Phill. *Brit. Discom.* p. 331; Rehm, *Discom.* p. 463; Sacc. *Syll. F.* VIII, 639 (No. 2634); Massee, *Brit. F. Fl.* IV, 151; Boud. *Discom. d'Eur.* p. 101.

Peziza fusarioides Berk. in *Mag. Zool. Bot.* I, 46 (No. 12); Berk. *Outl.* p. 371; Cke. *Handb.* p. 704 (No. 2114).

This species has always been interpreted correctly. A very full description is given by v. Höhnel (Fr. z. M. Nos. 1063–4).

7. ***Calycella claroflava*** (Grev.) Boud. *Discom. d'Eur.* p. 95.

Peziza claroflava Grev. *Fl. Edin.* p. 424; Berk. in *Engl. Fl.* V, pt. 2, p. 203.

Helotium claroflavum Berk. *Outl.* p. 372; Cke. *Handb.* p. 713 (No. 2150); Phill. *Brit. Discom.* p. 165; Sacc. *Syll. F.* VIII, 225 (No. 914); Massee, *Brit. F. Fl.* IV, 233.

In Herb. Kew. I saw what may be the specimen referred to by Massee, viz. one labelled in Cooke's handwriting: "*Helotium claroflavum*—ex Grev." This specimen, as well as numerous others both at Kew and in Brit. Mus., is typical young *Calycella citrina* (Hedw. ex Fr.) (= *Helotium citrinum* Fr.). In this connection the following note by Phillips is of a certain interest: "Most of my British specimens are immature" (in litt. ad Cooke, 18. v. 1876). The immaturity of the apothecia is evidently the cause of the small size of the spores.

8. ***Calycella flava*** (Klotzsch) Boud. *Discom. d'Eur.* p. 95.
Peziza flava Klotzsch in sched.
Helotium flavum Phill. *Brit. Discom.* p. 156; Sacc. *Syll. F.* VIII, 225 (No. 915); Massee, *Brit. F. Fl.* IV, 241.

The type specimen, which was very fully described by Massee, in my opinion represents typical *Calycella citrina* (Hedw. ex Fr.). It differs only externally by the somewhat horny, semitranslucent apothecia of a more uniform yellowish white. In winter, after frost, the apothecia of *C. citrina* undergo, however, just these changes. As to anatomical structure, asci and spores no differences could be detected.

9. ***Cudoniella Allenii*** A. L. Sm. in *Trans. Brit. mycol. Soc.* III, 40; Sacc. *Syll. F.* XXII, 603 (No. 5331).

The type specimen in Herb. Mus. Brit. is typical *Corynella atrovirens* (Pers.) Boud. The apothecia are remarkably well developed with convex hymenium, and their stemlike bases are somewhat more conspicuous than usual.

It will be shown below that *Pachydisca agaricina* (Carm. ap. Berk.) Boud. (note 30) and *Pithyella hydnicola* (B. & Br.) Boud. (note 39) are also *Corynella atrovirens*.

10. ***Dasyscypha campylotricha*** A. L. Sm. in *Trans. Brit. mycol. Soc.* III, 112; Sacc. *Syll. F.* XII, 684 (No. 5616) ("campylotrichia").

This species belongs to the genus *Unguiculella* v. Höhn. The specimens distributed by Karsten in F. Fenn. No. 652 (on *Artemisia vulgaris*) as *Peziza eurotioides* Karst. are the same fungus. It is evidently very rare, collected only in the type gatherings.* Its correct name is *Unguiculella eurotioides* (Karst.) Nannf. n.comb.

11. ***Dasyscypha crucifera*** (Phill.) Sacc. *Syll. F.* VIII, 440 (No. 1833); Massee, *Brit. F. Fl.* IV, 331; Boud, *Discom. d'Eur.* p. 119.
Peziza crucifera Phill. in *Gard. Chron.* (1878), p. 397.
Lachnella crucifera Phill. *Brit. Discom.* p. 250.

This species belongs—as the descriptions also bear witness—to the genus *Lachnum* Karst., and its correct name is *Lachnum cruciferum* (Phill.) Nannf. n.comb.

As far as I am aware, this fungus has never been reported from outside Britain, but I have seen it several times in Sweden and think it is common everywhere on *Myrica Gale*.

12. ***Discinella exidiiformis*** (B. & Br.) Boud. *Discom. d'Eur.* p. 96.
Peziza exidiiformis B. & Br. in *Ann. Mag. nat. Hist.* (4), XV, 37 (No. 1480); Cke. *Mycogr.* f. 60; Phill. *Brit. Discom.* p. 81; Massee in *J. linn. Soc.* (*Bot.*), XXXI, 501.

* My report of *Peziza eurotioides* from Sweden (*Svensk bot. Tidskr.* XXII, 131 as *Pezizella eurotioides*) is based upon an erroneous determination.

Humaria exidiiformis Sacc. *Syll. F.* VIII, 122 (No. 468); Massee, *Brit. F. Fl.* IV, 418.

This species belongs to the operculate Discomycetes, probably to the genus *Humaria* (=*Humarina* Seav.), as Saccardo and Massee (*Brit. F. Fl.*) suggested.

13. ***Encoelia Bloxami*** (Berk.) Phill. *Brit. Discom.* p. 338; Boud. *Discom. d'Eur.* p. 161.

Patellaria Bloxami Berk. in sched.
Cenangium Bloxami Sacc. *Syll. F.* VIII, 568 (No. 2343); Massee, *Brit. F. Fl.* IV, 114.

As noted already by Massee on the type specimen (at Kew), this species is identical with *Diplocarpa Curreyana* Massee.

14. ***Habrostictis lasia*** (B. & Br.) Boud. *Discom. d'Eur.* p. 102.

Peziza lasia B. & Br. in *Ann. Mag. nat. Hist.* (4), II, 347 (No. 1391).
Calloria lasia Phill. *Brit. Discom.* p. 327.
Orbilia lasia Sacc. *Syll. F.* VIII, 625 (No. 2574); Rehm, *Discom.* p. 456; Massee, *Brit. F. Fl.* IV, 146.

This species was treated earlier both by v. Höhnel (Fr. z. M. No. 1016) and myself (Nannfeldt, 1932, p. 97), but we did not study the actual type. The type specimen proves to be identical with the species treated by us and generally known as *Habrostictis lasia* (B. & Br.) Boud. or *H. rubra* Fuck. The latter is the valid name.

15. ***Hyalinia incarnata*** (Cke.) Boud. *Discom. d'Eur.* p. 103.

Peziza (*Mollisia*) *incarnata* Cke. in *Grev.* I, 131.
Mollisia incarnata Phill. *Brit. Discom.* p. 191; Massee, *Brit. F. Fl.* IV, 216.
Pezizella incarnata Sacc. *Syll. F.* VIII, 285 (No. 1186).

The type specimen (in Herb. Kew.) is in a rather bad condition and very poor, but it allows of the certain identification of this species as *Pseudohelotium pineti* (Batsch ex Fr.) Fuck. Further notes on this species are given above under *Belonidium Jerdoni* (Cke. & Phill.) Massee (note 1).

16. ***Lachnella canescens*** (Cke.) Phill. *Brit. Discom.* p. 259; Sacc. *Syll. F.* VIII, 394 (No. 1620); Boud. *Discom. d'Eur.* p. 123.

Peziza canescens Cke. in litt. ad Phill.
Dasyscypha canescens Massee, *Brit. F. Fl.* IV, 346.

Phillips placed this species next to *Lachnella corticalis* Fr. but considered it specifically distinct because of its somewhat different spores and "the more conspicuous septate hairs of the exterior". Examination of the type specimen (at Kew) proved that the alleged differences are very slight, if any, and fall within the variation shown by *L. corticalis*.

I have shown (Nannfeldt, 1932, p. 265) that the generic name *Lachnella* cannot be used in this sense, and that the species is a *Lachnum, L. corticale* (Pers. ex Fr.) Nannf.

17. ***Lachnella Crosslandi*** Boud. ap. Ramsb. in *Trans. Brit. mycol. Soc.* IV, 375.
Echinella Crosslandi Massee, *Brit. F. Fl.* IV, 306.
Pirottaea Crosslandi Sacc. in *Hedw.* XXXV, Beih. 7, p. xxxvi; Sacc. *Syll. F.* XIV, 776 (No. 2913).

The type specimen (at Kew) is *Lachnum corticale* (Pers. ex Fr.) Nannf. (see Nannfeldt, 1932, pp. 129 and 265) with unusually well-developed spores.

18. ***Lachnella (Helotiella) Laburni*** Phill. in *Scot. Nat.* (1891), p. 90; A. L. Smith in *Trans. Brit. mycol. Soc.* IV, 76.
Helotiella Laburni "A. L. Sm." Sacc. *Syll. F.* XXIV, 1209 (No. 7267).

The type specimen (at Brit. Mus.) is very poor. The only Discomycete that I could find on it was *Unguicularia scrupulosa* (Karst.) v. Höhn. Phillips's description matches it tolerably, except for the asci and the spores. It is impossible to tell whether he made mistakes when measuring them or studied a mixture of two species.

19. ***Lachnella setulosa*** (Massee & Crossl.) Boud. ap. Ramsb. in *Trans. Brit. mycol. Soc.* IV, 375.
Echinella setulosa Massee & Crossl. ap. Massee, *Brit. F. Fl.* IV, 305.
Pirottaea setulosa Sacc. in *Hedw.* XXXV, Beibl. 7, p. xxxvi; Sacc. *Syll. F.* XIV, 776 (No. 2912).

I have recently suggested that this species might be identical with *Trichobelonium obscurum* Rehm (Nannfeldt, 1932, p. 167). In Crossland's herbarium (now at Kew) there may be found not only the type collection but also seven additional gatherings. They are all identical with Rehm's species. *T. obscurum* Rehm is the valid name.

20. ***Lachnella siparia*** (B. & Br.) Phill. *Brit. Discom.* p. 276; Sacc. *Syll. F.* VIII, 396 (No. 1629); Boud. *Discom. d'Eur.* p. 123.
Peziza (*Fibrina*) *siparia* B. & Br. in *Ann. Mag. nat. Hist.* (2), XIII, 465 (No. 772); Berk. *Outl.* p. 370; Cke. *Handb.* p. 696 (No. 2079).
Dasyscypha siparia Massee, *Brit. F. Fl.* IV, 367.

The type (at Kew and Brit. Mus.) shows that *Peziza siparia* is a true *Encoelia* (Fr.) Karst. (see Nannfeldt, 1932, pp. 303–4) and identical with *Cenangium Ulmi* Tul. The British specimens are not—as Berkeley & Broome state—"on decorticated elm branches" but on

the inner bark of elm. As the specific epithet "*siparia*" antedates "*Ulmi*", the species should be known as *Encoelia siparia* (B. & Br.) Nannf. n.comb.

21. ***Lecanidion clavisporum*** (B. & Br.) Sacc. & D. Sacc. ap. Sacc. *Syll. F.* XVIII, 184 (No. 3808); Boud. *Discom. d'Eur.* p. 154.

Patellaria clavispora B. & Br. in *Ann. Mag. nat. Hist.* (2), XIII, 465 (No. 774); Berk. *Outl.* p. 373; Cke. *Handb.* p. 717 (No. 2166); Phill. *Brit. Discom.* p. 366; Massee, *Brit. F. Fl.* IV, 102; Massee in *J. linn. Soc.* (*Bot.*), XXXV, 107.

Durella clavispora Sacc. *Syll. F.* VIII, 794 (No. 3257).

Two authentic gatherings of this species were seen by me, viz. the type collection from Lucknam Grove (at Kew and Brit. Mus.) and a second gathering from St Catharine's (Herb. Broome in Brit. Mus.). They are both identical with *Lecanidion Crataegi* (Phill.) Sacc. (see next entry) and *Patellaria corticola* Starb.

I have published a photomicrograph of *P. corticola* and pointed out (Nannfeldt, 1932, p, 196) that it shows no relationship to the genus *Patellaria*. I know no other genus where it could be placed, and its affinity is still obscure to me.

22. ***Lecanidion Crataegi*** (Phill.) Sacc. *Syll. F.* VIII, 799 (No. 3276); Boud. *Discom. d'Eur.* p. 155.

Patellaria Crataegi Phill. in *Grev.* XVII, 46; Massee, *Brit. F. Fl.* IV, 106.

In Herb. Phillips (at Brit. Mus.) are preserved two specimens under this name, both collected by Trail. The type specimen ("No. 3—on hawthorn twigs—Corbie Den—1. ii. 89") is identical with *Lecanidion clavisporum* (B. & Br.) Sacc. & D. Sacc. (see preceding entry). The second specimen ("No. 15—on bramble—nr Aberdeen—4. iii. 87") is a long-spored *Durella*.

The Romell specimen (Sweden: Gotland, Visby, 1. vii. 1887) in Herb. Kew. to which Massee (*loc. cit.*) alludes, is also *Lecanidion clavisporum*.

23. ***Lecanidion Hyperici*** (Phill.) Sacc. *Syll. F.* VIII, 801 (No. 3288); Boud. *Discom. d'Eur.* p. 155.

Patellaria Hyperici Phill. in *Grev.* X, 69; Phill. *Brit. Discom.* p. 363; Massee, *Brit. F. Fl.* IV, 107.

This species, which is distributed in Phill. *Elv. Brit.* No. 191, is identical with *Durella atrocyanea* (Fr.) v. Höhn. (= *Stictis atrocyanea* Fr.). The apothecia are situated in greenish spots as they usually are in that species, though this feature was not mentioned in any description of *Patellaria Hyperici*. For further particulars about the very much misunderstood *Durella atrocyanea* see v. Höhnel, *Ann. mycol.*, Berl., XVI, 210–12.

24. ***Lecanidion Lonicerae*** (Phill.) Sacc. *Syll. F.* VIII, 797 (No. 3267); Boud. *Discom. d'Eur.* p. 155.

Patellaria Lonicerae Phill. *Brit. Discom.* p. 364; Massee, *Brit. F. Fl.* IV, 104.

No specimen, but only a drawing was to be found in Phillips's herbarium (at Brit. Mus.). A specimen in Cooke's herbarium (at Kew), labelled by Phillips "*Patellaria Lonicerae* n.s.—On honeysuckle—Darnaway, N.B." is evidently part of the type material. The fungus is a true *Durella*, which I am unable to separate from *D. vilis* Starb. As Phillips's name is the older, the valid name of the species is *D. Lonicerae* (Phill.) Nannf. n.comb. (see Nannfeldt, 1932, p. 293).

25. ***Mollisia atrocinerea*** (Cke.) Phill. *Brit. Discom.* p. 176; Sacc. *Syll. F.* VIII, 322 (No. 1334); Massee, *Brit. F. Fl.* IV, 208; Boud. *Discom. d'Eur.* p. 136 (non Rehm, *Discom.* p. 530).

Peziza atrocinerea Cke. *F. Brit. Exs.* Ed. ii, no. 382.

As both Massee (*loc. cit.*) and Morgan (*J. Mycol.* VIII, 182) have already suggested, this species, on *Polygonum*, is identical with the older *Mollisia Polygoni* (Lasch) Rehm. Rehm lists a *M. atrocinerea* (Cke.) Phill. which he regards as very closely allied to *M. atrata* (Pers. ex Fr.) Karst. and to *M. revincta* Karst. Rehm's species grows on stems of several different herbs but not on *Polygonum*. His herbarium shows that he placed numerous different fungi under this name as well as under *Mollisia atrata*.

M. Polygoni is a very characteristic species, differing in many respects from both *Mollisia* and *Pyrenopeziza*. Probably it should be placed in a separate genus of its own, but the time is not yet ripe for a definite system of Mollisioideae.

26. ***Mollisia Browniana*** (Blox. ap. B. & Br.) Sacc. *Syll. F.* VIII, 327 (No. 1355); Boud. *Discom. d'Eur.* p. 137.

Peziza (*Mollisia*) *Browniana* Blox. ap. B. & Br. in *Ann. Mag. nat. Hist.* (3), XV, 446 (No. 1072); Cke. *Handb.* p. 702 (No. 2102); Phill. *Brit. Discom.* p. 408.

Pseudopeziza Browniana Massee, *Brit. F. Fl.* IV, 199.

Examination of the type collection (at Kew and Brit. Mus.) proved that this species has been totally misinterpreted. The fungus is *Heterosphaeria Patella* Grev., and the substratum is not *Epilobium hirsutum* but some kind of Umbelliferous plant, almost certainly *Angelica silvestris*.

27. ***Niptera Stockii*** (Cke. & Phill.) Boud. *Discom. d'Eur.* p. 141.

Peziza Stockii Cke. & Phill. in sched.
Lachnella Stockii Phill. *Brit. Discom.* p. 261.
Belonium Stockii Sacc. *Syll. F.* VIII, 496 (No. 2048).
Echinella Stockii Massee, *Brit. F. Fl.* IV, 307.

The type specimen (at Kew) is in a very bad condition. It could be ascertained, however, that the fungus belongs to the genus *Pyrenopeziza* Fuck. emend. Nannf. It has septate spores and the margin of the excipulum passes into long, obtuse, hyaline hairs. The taxonomic position is next to *P. Arctii* (Phill.) Nannf. and *P. leucostoma* (Karst.) Nannf. Its identity must be left undecided until the host plant can be determined. It is probably a Composite.

28. ***Orbilia Boydii*** A. L. Sm. & Ramsb. in *Trans. Brit. mycol. Soc.* II, 168; Sacc. *Syll. F.* XXIV, 1239 (No. 7370).

The type specimen (in Herb. Brit. Mus.) as well as another authentic specimen ("on dead twigs of *Vaccinium Myrtillus*—Beith, Ayrshire—20th July 1912—D. A. Boyd") both show a very young, hardly ripe *Pezicula*, which can with certainty be identified with *P. myrtillina* Karst.

29. ***Orbilia scotica*** Massee, in *Grev.* XXII, 99; Massee, *Brit. F. Fl.* IV, 144; Sacc. *Syll. F.* XI, 426 (No. 2661); Boud. *Discom. d'Eur.* p. 103.

In the original description of this species Massee gives the information that it is based on a specimen from "Aboyne, N.B." lying in the Berkeley herbarium at Kew as *Peziza vinosa*. On a sheet of *Orbilia vinosa* there, I found a specimen marked in Berkeley's handwriting: "*Pez.* (*Mollisia*)—Aboyne, Sept. 1870." In my opinion this specimen must be the type, though it bears no note at all in Massee's hand. It matches the description of *Orbilia scotica* very well, except for the spores. These were extremely difficult to see, as they usually are in herbarium specimens of *Orbilia*, and the poorness of the material did not permit studying more than one apothecium. Nevertheless, I was able to find a few genuine free spores which were undoubtedly ascospores. They were needle-shaped and about $12 \times 1–1{\cdot}5\,\mu$. I have no hesitation in saying that Massee's description of the spores as "elliptic-oblong, ends obtuse, $4 \times 1\,\mu$" was due to faulty observation. *O. scotica* is identical with the species that W. Nylander (*Not. Sällsk. F. Fl. Fenn. Förh.* x, 56) described as *Peziza vinosa* A. & S. and that has later been known as *Orbilia vinosa* (A. & S.) Karst. It is a typical *Orbilia*, but it is impossible to decide whether it is identical with the original *Peziza vinosa* of Albertini & Schweinitz.

30. ***Pachydisca agaricina*** (Carm. ap. Berk.) Boud. *Discom. d'Eur.* p. 93.
Peziza agaricina Carm. ap. Berk. in *Engl. Fl.* V, pt. 2, p. 207.
Helotium agaricinum Berk. *Outl.* p. 371; Cke. *Handb.* p. 708 (No. 2127); Sacc. *Syll. F.* VIII, 220 (No. 896).
Belonidium agaricinum Massee, *Brit. F. Fl.* IV, 224.

The type specimen (in Herb. Kew.) proved to be *Corynella atrovirens* (Pers.) Boud.

31. ***Pachydisca brunnea*** (Phill.) Boud. *Discom. d'Eur.* p. 94.
Ombrophila brunnea Phill. in *Grev.* VIII, 103; Phill. *Brit. Discom.* p. 323; Sacc. *Syll. F.* VIII, 619 (No. 2551); Massee, *Brit. F. Fl.* IV, 143.

This species belongs to the operculates and probably to the genus *Humaria* (= *Humarina* Seav.), but nothing definite about its identity can be said for the present.

32. ***Pachydisca Laburni*** (B. & Br.) Boud. *Discom. d'Eur.* p. 94.
Helotium Laburni B. & Br. in *Ann. Mag. nat. Hist.* (4), XVII, 143 (No. 1624); Sacc. *Syll. F.* VIII, 249 (No. 1027); Massee, *Brit. F. Fl.* IV, 235; Massee in *J. linn. Soc.* (*Bot.*), XXXI, 475.
Hymenoscypha Laburni Phill. *Brit. Discom.* p. 135.

Rehm (*Discom.* p. 787) identified this species with the older *Helotium infarciens* Ces. & deNot. This identification is correct.

33. ***Pachydisca ochracea*** (Grev.) Boud. *Discom. d'Eur.* p. 93.
Peziza ochracea Grev. *Scott. Crypt. Fl.* pl. 5; Berk. *Engl. Fl.* V, pt. 2, p. 204.
Helotium ochraceum Berk. *Outl.* p. 372; Cke. *Handb.* p. 713 (No. 2148); Phill. *Brit. Discom.* p. 169; Sacc. *Syll. F.* VIII, 229 (No. 937); Massee, *Brit. F. Fl.* IV, 237.

The type of this species is evidently lost, and Massee based his description on the original one, adding microscopical details from a Carmichael specimen (determined by Klotzsch) in Herb. Kew. This specimen is still there, but I could detect no Discomycete on it. If one may make a guess from Greville's drawing and description, I should suggest that they represent some species of *Pezicula*, tentatively *P. livida* (B. & Br.) Rehm. The description of the hymenium "as if sprinkled with minute shining particles not unlike small grains of brown sugar", strongly suggests that genus (see Nannfeldt, 1932, p. 90).

Massee's description of the microscopical features indicates that the Carmichael specimen also belonged to the genus *Pezicula*.

Specimens in Herb. Brit. Mus. from Broome's herbarium marked "*Peziza ochracea*—Hartham Park—21. iii. 43", are old *Calycella citrina* (Hedw. ex Fr.).

34. ***Pachydisca quisquilaris*** (Phill.) Boud. *Discom. d'Eur.* p. 94.

No such species was ever described, Boudier's reference "*quisquilaris* Phill., *Grev.* XVI, p. 94—Sacc. *Syll. F.* VIII, p. 617" being a mixture of *Helotium quisquiliaris* Karst. and *Ombrophila helotioides* Phill.

35. ***Pachydisca scoparia*** (Cke.) Boud. *Discom. d'Eur.* p. 94.
Helotium scoparium Cke. in *Grev.* IV, 111; Phill. *Brit. Discom.* p. 168; Sacc. *Syll. F.* VIII, 239 (No. 974); Massee, *Brit. F. Fl.* IV, 234.

The type specimen at Kew represents a typical *Pezicula Rubi* (Lib.) Niessl, but the apothecia have (from age or bad preservation) lost most of their characteristic colour. It proved, on microscopical examination, to be unchanged internally.

36. ***Patinella Euphorbiae*** (B. & Br.) Sacc. *Syll. F.* VIII, 771 (No. 3171); Boud. *Discom. d'Eur.* p. 146.
Peziza (*Patella*) *Euphorbiae* B. & Br. in *Ann. Mag. nat. Hist.* (5), III, 212 (No. 1829).
Mollisia Euphorbiae Phill. *Brit. Discom.* p. 198.
Pseudopeziza Euphorbiae Massee, *Brit. F. Fl.* IV, 197.

I have studied the type specimen in Herb. Brit. Mus. The species proves to be identical with *Naevia tithymalina* (J. Kze.) Rehm (=*Calloria tithymalina* J. Kze.). The material is old and in bad condition; hence the dark colour.

Kunze's specific epithet antedates "*Euphorbiae*" by three years. The correct taxonomic position of the fungus is not clear, though I believe it to be closely related to my genus *Laetinaevia* (see Nannfeldt, 1932, pp. 190–2).

37. ***Peristomialis Berkeleyi*** Boud. *Discom. d'Eur.* p. 116.
Peziza (*Mollisia*) *peristomialis* B. & Br. in *Ann. Mag. nat. Hist.* (3), XVIII, 126 (No. 1169); Cke. *Handb.* p. 706 (No. 2119); Massee in *J. linn. Soc.* (*Bot.*), XXXV, 99.
Mollisia peristomialis Phill. *Brit. Discom.* p. 201.
Cyathicula peristomialis Sacc. *Syll. F.* VIII, 308 (No. 1284); Massee, *Brit. F. Fl.* IV, 273 ("*peristomalis*").

This very curious species was the only member of the subgenus *Peristomialis* of *Mollisia* in Phillips's *Brit. Discom.*, and Boudier later established the genus *Peristomialis*, changing the specific name of the species into *P. Berkeleyi*. The taxonomic position of the genus was discussed by v. Höhnel (M. Fr. p. clviii), and he suggested from the description and the illustrations that it might belong to the "Nectriaceae". When studying the type specimen I recognised at once that v. Höhnel had been right in excluding the genus from the Discomycetes and that, in reality, it is identical with *Ijuhya* Starb. V. Höhnel has given several good descriptions of this hitherto monotypic genus (*Denkschr. Akad. Wiss. Wien*, LXXXIII, 22; Fr. z. M. pp. 691 and 762). I cannot for the present decide whether *Peziza peristomialis* is identical

with *Ijuhya vitrea* Starb., known from Brazil and Java, or specifically distinct from it.

38. ***Phaeangium phaeosporum*** (Cke.) Sacc. & Syd. ap. Sacc. *Syll. F.* xvi, 765; Boud. *Discom. d'Eur.* p. 162.
Cenangium phaeosporum Cke. in *Grev.* xii, 44; Phill. *Brit. Discom.* p. 346; Sacc. *Syll. F.* viii, 570 (No. 2354).
Schweinitzia phaeospora Massee, *Brit. F. Fl.* iv, 135.

The type specimen in Herb. Kew. is poor and in a very bad condition. No character could be detected that would distinguish it from *Velutaria rufo-olivacea* (A. & S. ex Fr.) Fuck. except the more uniform darker colours, which are certainly due to bad preservation. Thus both the original species of *Schweinitzia* Massee (non Elliot) are *Velutaria rufo-olivacea* and Massee's genus becomes synonymous with *Velutaria* Fuck. (see Nannfeldt, 1932, p. 302).

Saccardo & Sydow placed *Cenangium phaeosporum* in the genus *Phaeangium* (Sacc.) Sacc. & Syd. (non Pat.). The first species of that genus, *P. Rubi* (Bäuml.) Sacc. & Syd., is most probably a synonym for *Pezicula Rubi* (Lib.) Niessl (see Nannfeldt, 1932, p. 92), and the sixth and last of the original species, *Phaeangium patellatum* (Cke.) Sacc. & Syd. (=*Cenangium patellatum* Cke.), described from U.S.A. as growing on branches of *Acer*, is, according to the type specimen (at Kew), *Dermatea Cerasi* Fr. on *Prunus*. It seems most probable that the remaining species of *Phaeangium* Sacc. & Syd. are stages of well-known species with spores coloured because of age or bad preservation.

The genus *Phaeangella* (Sacc.) Massee seems to be in a similar position. Two species described by Hazslinsky as *Cenangium quercinum* (on oak) and *Tympanis Potentillae* (on *Potentilla fruticosa*) and later transferred to *Phaeangella* are both according to authentic material at Kew *Dermatea Cerasi* Fr. (on *Prunus*!). It is almost incredible how careless mycologists have been in describing "new" species and in naming their host plants. *Phaeangella Prunastri* (Fr.) Massee and *P. Morthieri* (Fuck.) Sacc. & Syd. are also species of *Dermatea*.

39. ***Pithyella hydnicola*** (B. & Br.) Boud. *Discom. d'Eur.* p. 125.
Peziza (*Mollisia*) *hydnicola* B. & Br. in *Ann. Mag. nat. Hist.* (4), vii, 434 (No. 1327).
Mollisia (*Mollisiella*) *hydnicola* Phill. *Brit. Discom.* p. 194.
Pseudohelotium (*Mollisiella*) *hydnicola* Sacc. *Syll. F.* viii, 304 (No. 1269) ("*hydnicolum*").
Mollisiella hydnicola Massee, *Brit. F. Fl.* iv, 223.

This species, which has remained very doubtful and known only from the very short original description, is represented in Broome's

herbarium (at Brit. Mus.) by part of the type gathering. Very superficial inspection showed that it did not grow *on* a *Hydnum*, but *through* a resupinate *Hydnum*. Its substratum is some kind of frondiferous wood, most probably oak. The fungus is typical *Corynella atrovirens* (Pers.) Boud. The spore description by Berkeley & Broome is totally false. They probably never saw the spores but mistook large drops of oil or protoplasm for them.

40. ***Pseudopeziza foecunda*** (Phill.) Massee, *Brit. F. Fl.* IV, 200; Boud. *Discom. d'Eur.* p. 180.
Peziza (*Mollisia*) *foecunda* Phill. ap. Stevenson, *Mycol. Scot.* p. 326.
Mollisia foecunda Phill. *Brit. Discom.* p. 189.
Pyrenopeziza foecunda Sacc. *Syll. F.* VIII, 369 (No. 1523).

This species, which was distributed in Phill. *Elv. brit.* No. 184, is identical with *Hysteropezizella subsessilis* (Rehm) Nannf. (see Nannfeldt, 1932, p. 121, where the full synonymy is to be found). The substratum given as "*Eleocharis*", is *Scirpus caespitosus*. As Phillips's name antedates the other names the fungus should be known as *Hysteropezizella foecunda* (Phill.) Nannf. n.comb.

Mollisia scirpina Peck (on *Scirpus caespitosus*) is certainly identical, but I know it only from the description.

41. ***Pyrenopeziza cyanites*** (Cke. & Phill.) Boud. *Discom. d'Eur.* p. 133.
Mollisia cyanites Cke. & Phill. ap. Phill. *Brit. Discom.* p. 176; Sacc. *Syll. F.* VIII, 351 (No. 1452).
Belonidium cyanites Massee, *Brit. F. Fl.* IV, 225.

The type specimen of this species (at Kew) is very poor. The substratum was originally given as "some herbaceous stem". I was therefore very surprised to recognise it as *Phragmites communis*. The fungus was so sparse that I had to refrain from making a microscopical study. The macroscopical features and Massee's very full description indicate *Tapesia Kneifii* (Wallr.) v. Höhn. (= *T. retincola* (Rabenh.) Karst.) (see v. Höhnel, Fr. z. M. p. 1223). It is most unfortunate that mycologists so often describe "new" species on such insufficient material and that they take so little care in naming the host plants!

Mollisia mediella Karst. may be the same species. The superficial Mollisioid Discomycetes on *Phragmites* are greatly in need of a critical revision. Cultural experiments are needed to show to what extent the development of a dark subiculum depends upon external conditions.

42. ***Pyrenopeziza grisella*** (Cke. & Phill.) Boud. *Discom. d'Eur.* p. 134.
Peziza grisea Carm. in sched.
Lachnella grisella Cke. & Phill. ap. Phill. *Brit. Discom.* p. 260.
Trichopeziza grisella Sacc. *Syll. F.* VIII, 413 (No. 1702).
Dasyscypha Carmichaeli Massee, *Brit. F. Fl.* IV, 363.

This species, according to the type specimen at Kew, belongs to *Unguicularia*. The hairs and the hymenium, etc., are exactly those of *U. scrupulosa* (Karst.) v. Höhn., but the apothecia are slightly larger than usual and the excipulum is remarkably dark, which may be due to age. We may safely regard the two species as synonymous. Karsten's specific epithet is the valid one.

43. ***Scleroderris majuscula*** Cke. & Massee, in *Grev.* XXI, 73; Massee, *Brit. F. Fl.* p. 125; Sacc. *Syll. F.* XI, 425 (No. 2652); Boud. *Discom. d'Eur.* p. 164.

The type specimen (at Kew) is very poor, consisting of a single, detached apothecium. It is *Coryne sarcoides* (Jacq. ex Fr.) Chev.

44. ***Tapesia Johnstoni*** (Berk.) Phill. *Brit. Discom.* p. 282; Sacc. *Syll. F.* VIII, 381 (No. 1570); Boud. *Discom. d'Eur.* p. 140.
Peziza Johnstoni Berk. in *Ann. Mag. nat. Hist.* (1), XIII, 17 (No. 313); Berk. *Outl.* p. 369; Cke. *Handb.* p. 695 (No. 2075); Massee in *J. linn. Soc.* (*Bot.*), XXXI, 515.

After examining the type material of *Peziza Johnstoni*, Massee (*loc. cit.*) identified it with *Tapesia fusca* (Pers. ex Fr.) Fuck. I was very surprised when I saw the material at Kew to find that Massee had been totally wrong, for *Peziza Johnstoni* is identical with the fungus known as *Cenangella radulicola* (Fuck.) Rehm (= *Cenangium radulicola* Fuck. = *Dermatea radulicola* Fuck.) growing, as always, on branches of birch infested with *Eutypa aterrima* (Fr.) v. Höhn. (= *Radulum aterrimum* Fr.). Berkeley's name antedates Fuckel's.

As I have not yet found appropriate material in quantity I have not been able to study the species in detail and cannot decide its taxonomic position.

45. ***Trichoscypha calycina*** var. ***Trevelyani*** (Cke.) Boud. *Discom. d'Eur.* p. 125 ("Trevyliani").
Peziza calycina var. *Trevelyani* Cke. in *Grev.* III, 121.
Lachnella calycina var. *Trevelyani* Phill. *Brit. Discom.* p. 242.
Dasyscypha calycina var. *Trevelyani* Sacc. *Syll. F.* VIII, 438 (sub No. 1822); Massee, *Brit. F. Fl.* IV, 342.

The type specimen of this variety is *Trichoscyphella Willkommii* (Hart.) Nannf. (= *Dasyscypha Willkommii* (Hart.)). I could not find any spores larger than $24 \times 8 \mu$, and the average size was $20 \times 7 \mu$,

just as in typical *Trichoscyphella Willkommii*. The larger measurements given by Cooke must be due to some mistake.

46. ***Trichopeziza dematiicola*** (B. & Br.) Sacc. *Syll. F.* VIII, 414 (No. 1707); Boud. *Discom. d'Eur.* p. 131.

Peziza (*Mollisia*) *dematiicola* B. & Br. in *Ann. Mag. nat. Hist.* (3), XV, p. 446 (No. 1070); Cke. *Handb.* p. 705 (No. 2117).
Lachnella dematiicola Phill. *Brit. Discom.* p. 265.
Dasyscypha dematiicola Massee, *Brit. F. Fl.* IV, p. 364 (saltem p.p.).
Dasyscypha dematiicola v. Höhn. in *S.B. Akad. Wiss. Wien*, CXVIII, Abt. 1, p. 884.

The type specimen (at Kew) was studied by v. Höhnel (Fr. z. M. p. 339), who identified it with his *Dasyscypha Heimerlii* v. Höhn. I have studied the type at Brit. Mus. and can only assert that it matches v. Höhnel's description of *D. Heimerlii* in all respects.

The systematic position of this species is somewhat obscure. The texture of the excipulum and the characteristic pointed hairs would place it in *Hyaloscypha* Boud. emend. Nannf. but the brown colour of the excipulum (the hairs and the margin excepted) distinguish it from the known species of that genus. Nevertheless, I think it best for the present to call it *Hyaloscypha dematiicola* (B. & Br.) Nannf. n.comb. (see Nannfeldt, 1932, p. 272).

47. ***Trochila Buxi*** Capron ap. Cke. *Handb.* p. 768 (No. 2315); Phill. *Brit. Discom.* p. 397; Sacc. *Syll. F.* VIII, 729 (No. 2991); Massee, *Brit. F. Fl.* IV, 61; Boud. *Discom. d'Eur.* p. 166.

The type specimen (Herb. Kew.) as well as two additional British specimens (Forden, leg. Vize, and Bungay, leg. Stock) are all *Hyponectria Buxi* (DC.) Sacc. A very full description of this species was given by Petrak (*Ann. mycol.* XXI, 303–6), who also discusses its taxonomic position. It may be noted that *Laestadia Buxi* (Fuck.) Sacc. is the same species (v. Höhnel, M. Fr. p. cci), as is also the fungus that Feltgen reported as *Trochila Buxi* Capron (v. Höhnel, *S.B. Akad. Wiss. Wien*, CXV, Abt. 1, p. 1263).

48. ***Urceolella elaphines*** (B. & Br.) Boud. *Discom. d'Eur.* p. 129.

Peziza elaphines B. & Br. in *Ann. Mag. nat. Hist.* (4), VII, 434 (No. 1325); Massee in *J. linn. Soc.* (*Bot.*), XXXV, 90.
Mollisia elaphines Gill. *Champ. Franc. Discom.* p. 131; Phill. *Brit. Discom.* p. 179.
Pseudohelotium elaphines Sacc. *Syll. F.* VIII, 301 (No. 1257).
Dasyscypha elaphines Massee, *Brit. F. Fl.* IV, 366.

The identity of *Peziza elaphines* B. & Br. and *Peziza scrupulosa* Karst. (= *Unguicularia scrupulosa* (Karst.) v. Höhn.) was demonstrated by

v. Höhnel (*Mitt. Bot. Inst. Techn. Hochsch. Wien*, v). I can only confirm v. Höhnel's statement.

49. ***Urceolella leuconica*** (Cke.) Boud. *Discom. d'Eur.* p. 130.
Peziza leuconica Cke. in sched.
Lachnella leuconica Phill. *Brit. Discom.* p. 267.
Trichopeziza leuconica Sacc. *Syll. F.* VIII, 414 (No. 1709).
Dasyscypha leuconica Massee, *Brit. F. Fl.* IV, 334.

The type specimen (at Kew) shows that it belongs to the genus *Hyaloscypha* Boud. emend. Nannf. (Nannfeldt, 1932, pp. 272–3.). The substrate is coniferous wood, probably pine. It is distinct from all species known to me by the much longer hairs. As closely allied forms are described in *Trichopeziza*, *Lachnella*, *Dasyscypha*, *Pezizella*, and many other genera, it is impossible for the present to form a definite opinion as to the validity of the specific epithet, but the species may *ad interim* be designated as *Hyaloscypha leuconica* (Cke.) Nannf. n.comb.

50. ***Urceolella Stevensonii*** (B. & Br.) Boud. *Discom. d'Eur.* p. 130 ("*Stephensoni*").
Peziza (*Mollisia*) *Stevensoni* B. & Br. in *Ann. Mag. nat. Hist.* (4), XV, 38 (No. 1485).
Lachnella Stevensoni Phill. *Brit. Discom.* p. 235.
Dasyscypha Stevensoni Sacc. *Syll. F.* VIII, 454 (No. 1889); Massee, *Brit. F. Fl.* IV, 364; Boud. *Discom. d'Eur.* p. 122.

Quite twenty years ago v. Höhnel (M. Fr. p. x) described *Dasyscypha resinifera* v. Höhn., a species said to grow on old, prostrate, still hard coniferous logs, and to be very common in Lower Austria, and also to occur in Germany and Sweden. I was later able to show (Nannfeldt, 1932, p. 273) that this most characteristic species was identical with *Pezizella atomaria* Starb. and belonged to *Hyaloscypha* Boud. emend. Nannf. During my stay in England I found that the fungus was described still earlier by Berkeley & Broome as *Peziza Stevensoni*, and that the substratum of the type material was pinewood. The valid name of this species therefore becomes *Hyaloscypha Stevensoni* (B. & Br.) Nannf. n.comb.

A specimen in Broome's herbarium (Brit. Mus.) is by a slip of the pen labelled *Peziza Andersoni*, and the collector's name given as J. Anderson.

A LEAF-SPOT DISEASE OF SWEET WILLIAM CAUSED BY *HETEROSPORIUM ECHINULATUM*

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(With 3 Text-figures)

Introduction

The diseased Sweet William plants (*Dianthus barbatus* L.) which were utilised in the present investigation were sent to the Plant Pathological Laboratory of the Department of Agriculture for Scotland in May 1932.

The leaves of the plants were heavily infected, bearing numerous brownish patches covered with olive-green conidia. The fungus was identified as *Heterosporium echinulatum* (Berk.) Cooke, and it was associated with some small black bodies on several of the over-wintered leaves. On examination a few of these bodies were found to contain mature asci, so, in view of the fact that no perfect stage of *Heterosporium echinulatum* has yet been recorded, the present investigation was undertaken primarily in order to ascertain whether these perithecia represented the perfect stage in the life history of this fungus. In the course of the work, however, certain other observations were made which have also been included in the present paper.

Historical

The fungus was first described and named in 1870 by Berkeley in an illustrated account of a new disease on carnation leaves. He placed the causal fungus in the genus *Helminthosporium* as a new species *H. echinulatum*.

In 1873 Berkeley & Broome published a description of a disease on Sweet William caused by another new species of *Helminthosporium*, *H. exasperatum*. Four years later Cooke transferred *H. echinulatum* to the genus *Heterosporium*, and cited *Helminthosporium exasperatum* as a synonym.

In 1881 Saccardo and Roumeguère described a fungus on *Dianthus barbatus* and named it *Heterosporium Dianthi*, but this name, together with *H. exasperatum*, was later cited by Saccardo (10) as a synonym of *H. echinulatum*.

In most references to the disease the parasite appears to have been confined to the leaves and stems of the hosts, but in 1890 Lindemuth

referred to a case where the mycelium spread to the sepals and prevented the opening of the flowers.

No mention is made of sclerotia in any of the earlier references to the disease, but in 1906 Cooke stated that numerous minute sclerotia are said to be formed in the dying leaves. Massee (8) also mentioned sclerotia, and recorded that they remain in a passive condition until the following season when they produce minute conidia. From this paucity of references to sclerotia in early records of the fungus it would seem that they are not of frequent occurrence.

Cultural studies

Ascospores were removed from crushed perithecia and placed in hanging drops of sterile water in a moist chamber. They germinated in less than twenty-four hours, each cell giving rise to a germ tube which grew rapidly and produced a branching mycelium in two to three days. In five to six days conidiophores developed which cut off typical *Heterosporium echinulatum* conidia, thus establishing the relation between the perfect and imperfect stage of this fungus. Single ascospores planted on malt extract agar gave rise to a somewhat dome-shaped mass of mycelium, white above and olive green underneath, which produced typical *H. echinulatum* conidia in four to five weeks when the colony was about 1–1¼ in. long.

Single conidia were also isolated from the original material and were found to give rise to two distinct types of culture on malt agar. One (type A) was exactly similar to that derived from single ascospores, the other (type B) differed in the time that elapsed before the formation of conidia, which here was only four to ten days. When conidia were produced the surface colour of the culture changed, becoming greyish green in type A and olive green in type B. This difference in colour appeared to be due to the development of large numbers of conidia on a small culture in type B whereas they were more scattered in type A. Conidia from these two types were repeatedly reisolated and transplanted but the distinction persisted on malt agar. If, however, sterilised carnation and Sweet William leaves were utilised instead of an agar medium, conidia were produced freely by both A and B types after ten to twelve days.

There appeared to be a great deal of variation in size between conidia from different cultures on the same medium and also between those on different media. Conidia from sterilised carnation leaf cultures, which were subcultures of the original malt agar cultures, were markedly larger than those of the parent cultures, and conidia from these leaf cultures put back on malt agar produced a mycelium which also gave rise to large conidia (Table I).

In all the malt agar cultures, whether derived from conidia or

Table I. *Measurements of conidia (in μ)*

Source of conidia	Range	Mean
Original material	16–52 × 8–14	31·71 × 10·81
Malt agar culture (A type) old	18–55 × 8–13	30·3 × 10·47
,, (B type) ,,	18–51 × 8–14	30·86 × 10·86
,, (A type) young	16–45 × 7–13	27·53 × 10·09
,, (B type) ,,	20–50 × 6–13	31·66 × 9·5
Sterilised carnation leaf culture	26–54 × 9–14	38·42 × 11·01
Subculture from carnation leaf on malt agar, 6 days old	29–53 × 9–14	39·93 × 12·37

ascospores, dark knotted masses of hyphae appeared after about five weeks. These were thought to be young perithecia, but they did not develop further although the cultures were kept for six months. On sterilised carnation and Sweet William leaves immature perithecia developed after twelve to fifteen days, the majority of them being formed on the surface furthest from the point of inoculation. They were most abundant towards the base of the tube where there was a greater percentage of water, and they developed particularly at points where the leaf was in contact with the glass. Perithecia in fifteen days old cultures measured from 60 to 116 μ in diameter, were black, roughly spherical, but differed from those of the original material in having a large number of black hyphae projecting from the walls. A short typical beak was present and the apical cells of the beak were colourless. On crushing them no asci were seen, and the contents were found to consist of a ball of hyaline cells and numerous oil globules.

In an attempt to obtain mature perithecia some conidia were planted on sterilised Sweet William leaves on December 6, 1932, and kept in the laboratory until January 10, 1933, when two of them were placed in an unheated greenhouse. Perithecia were taken from all the cultures and examined every fortnight, and asci were found in the greenhouse cultures in May 1933. The percentage of perithecia containing asci was small, as in the original material, and many of the asci were degenerate, but a few contained fully developed ascospores which agreed in every respect with those found on the original material. None of the perithecia in cultures kept in the laboratory developed asci, but the perithecia in seven to eight months old cultures which were kept damp grew very irregular in shape and the beak often became elongated (Fig. 1 *b*). In cultures which were allowed to dry the perithecia remained spherical and exactly resembled those of the original material.

Description of fungus

The conidial stage has been frequently described (1, 2, 3, 8), and it is therefore proposed to proceed at once to the description of the perfect stage.

The perithecia were black and irregularly spherical, 100–270 μ in diameter, and possessed short stout beaks 10–30 μ high (Fig. 1 *a*). The outer part of the walls consisted of two to three layers of dark brown cells, and the inner part of a few layers of colourless cells.

Fig. 1. *Didymellina Dianthi*. A, typical perithecium on original diseased material; B, abnormal perithecium from culture on Sweet William leaf. Perithecia × 400.

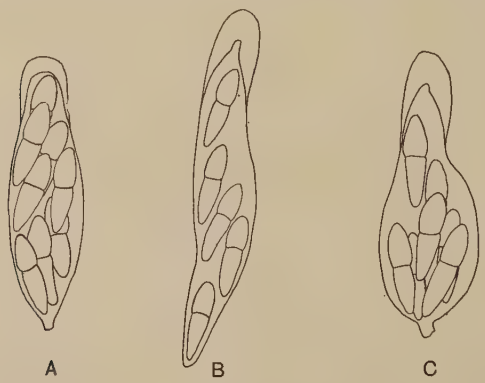

Fig. 2. *Didymellina Dianthi*. Asci from Sweet William leaf culture. A, typical ascus from centre of perithecium; B, abnormally elongated ascus; C, ascus from side of perithecium. (× 500.)

The percentage of perithecia containing asci was small, about 10 per cent., and the remainder appeared to be sterile. When kept under moist conditions, however, many of the sterile perithecia developed tufts of conidiophores from the beak, the conidia exactly

resembling those of *Heterosporium echinulatum*. None of the fertile perithecia examined were producing conidia, so it would appear that sterile perithecia may function as sclerotia. This resembles the behaviour reported by Tisdale in the related species *H. gracile* (11).

The number of asci in a fertile perithecium varied from eight to eighteen according to the size of the perithecium. They were fascicled and attached by short pedicels to a mass of pseudoparenchyma in the base of the perithecium. Paraphyses were absent. The asci were thin-walled, hyaline and very irregular in shape. As the bundle of asci fitted exactly into the cavity of the perithecium, those towards the outside were shorter, stouter and club-shaped (Fig. 2 *c*), whereas those in the centre tended to be elongated and spindle-shaped (Fig. 2 *a*, *b*). The wall was thickened at the apex up to 10 μ, and occasionally the cavity of the ascus was extended upwards into the centre of this thickening (Fig. 2 *b*, *c*).

Most of the asci contained eight ascospores lying parallel or slightly oblique to the long axis of the ascus. The ascospores measured 22–31 × 7–9 μ, and were torpedo-shaped, two-celled, thin-walled and colourless, the upper cell being 1–6 μ (average 3·3 μ) shorter, and slightly wider than the basal cell. The spores were somewhat constricted at the septum.

Infection experiments

Infection experiments were carried out with pot plants of Sweet William, carnations and pinks. A suspension of conidia was injected into the leaves with a hypodermic syringe; the control plants were similarly treated, sterile water being used instead of the suspension. The conidia for some inoculations were obtained from monoascospore cultures, for others from type A or B cultures derived from single conidia off the original material. Ascospores were not utilised for these experiments owing to the scarcity of mature asci. The plants were kept under bell jars in the laboratory for forty-eight hours after inoculation and then placed in an unheated greenhouse and watered from below.

All the Sweet William plants developed lesions around the point of inoculation. These consisted of yellowish brown withered patches with a purplish margin (Fig. 3); they appeared in six to ten days and attained a diameter of about half an inch after four weeks. Very few conidia were found on these patches, so, since extremely dry conditions were prevalent in the greenhouse, an infected leaf was removed and placed on moist filter paper in a sterile Petri dish. Numerous hyphae developed within twenty-four hours on both sides of the infected area, and it was green with conidia forty-eight hours after removal to moist conditions. One of the infected plants was then placed under a bell jar, and it developed numerous conidia on the

infected areas after forty-eight hours. Conidia from these leaves were isolated on malt agar slopes and gave rise to typical cultures. The uninfected leaves of the plants remained healthy and the plants flowered.

Two months after inoculation the diseased areas had extended over the greater part of the infected leaves, but the plants were otherwise healthy. The control plants remained healthy and the injured points healed leaving only a small yellow scar.

Fig. 3. Sweet William leaf artificially injected with *Heterosporium echinulatum* photographed two months after inoculation. The upper half of the leaf has been killed and the diseased area is delimited by a dark purple margin. About two-thirds natural size.

The injected carnations and pinks, on the other hand, showed only a very slight reaction, consisting of a yellowish area with a definite purple margin which, after four weeks, extended about $\frac{1}{8}$ in. around the point of inoculation. No *Heterosporium* conidia were found on the infected leaves, but in one or two sections hyphae were found on the injured part of the epidermis apparently growing saprophytically. Two leaves of pink and two of carnation were therefore cut off and kept moist in sterile Petri dishes. In two days hyphae developed on both of the pink leaves and on one of the carnation leaves, and conidia appeared in three days. These were *Cladosporium* on both pink leaves and on one of the carnations, but on the other carnation a few *Heterosporium echinulatum* conidia were also found. The mycelium,

however, did not spread to the uninjured part of the leaf which remained free from hyphae.

The wound made by the needle on the controls healed, leaving a small yellow withered piece of epidermis, but no extension of the necrotic area was observed and no purple discoloration developed.

Table II. *Infection experiments*

Host	No. of plants	Total no. of leaves inoculated	Result		No. of control plants	No. of leaves injected	Result	
			Diseased	Healthy			Diseased	Healthy
Sweet William	3	22	20	2	1	6	0	6
Carnation	6	36	32*	4	1	6	0	6
Pink	3	24	20*	4	1	6	0	6

* Slightly discoloured.

The results of the infection experiments are summarised in Table II. These inoculations were all carried out in early summer, but a subsequent series made on Sweet William in the autumn showed that infection did not take place so readily at that season, neither did the lesions attain so great a size.

It is evident that the strain of *Heterosporium echinulatum* utilised in the present investigation is actively parasitic on Sweet William but has little effect on carnations and pinks. The two last-named plants, however, are well known as hosts of this fungus, a fact which leads to the conclusion that there must be two or more specialised races of the fungus upon the different host plants.

Discussion

There appears to be no previous record of the perfect stage of *Heterosporium echinulatum*, nor could I find a description of any perithecial form on Sweet William, carnation or pink which corresponds to the perithecial stage described in the present paper. Tisdale (11) and Klebahn (6) have both published accounts of the perfect stage of *H. gracile*, the former identifying it as *Didymellina Iridis* (Desm.) v. H., while the latter regarded it as a new species which he named *D. macrospora*. The genus *Didymellina* was established by von Höhnel (5), but no adequate generic description was published. This fact somewhat complicates the identification of the perfect stage of *Heterosporium echinulatum*, but as the fungus agrees in essential points with the species described by Tisdale, it is thought advisable to use the name *Didymellina* for the perithecial form described in the foregoing pages. The specific epithet *echinulatum* refers to a character of the conidium wall which is by no means always well marked and which, in any case, is not applicable to the ascospores. The name *Didymellina*

Dianthi is therefore proposed for the new perfect stage of the fungus, since it occurs only, so far as we know, upon certain species of *Dianthus*.

Didymellina Dianthi n.sp.

Perithecia suberumpentia, atrobrunnea vel nigra, laxe gregaria, irregulariter sphaerica, 100–270 μ diametro, rostro brevi 10–30 μ longo. Asci in perithecio 8–18-nati, fasciculati, breviter pedicellati, quoad formam atque magnitudinem valde variabiles, muro hyalino infra tenui apicem versus ad 10 μ crasso, sporis octonis; paraphyses desunt. Ascosporae hyalinae, ovali-ellipticae, 22–31 × 7–9 μ, uniseptatae, ad septum paulo constrictae, cellula superiore paululo breviore atque latiore quam inferiore.

Hab. in foliis *Dianthi barbati* tempore hiberno emarcidis. Apud hortos nonnullos in Scotia.

Forma conidialis—*Heterosporium echinulatum* (Berk.) Cooke.

Summary

1. The perfect stage of *Heterosporium echinulatum* was found on some over-wintered leaves of diseased Sweet William plants, and was subsequently obtained in culture on sterilised Sweet William leaves. The relation between the two stages was proved by monospore cultures.

2. Only a small percentage of the perithecia matured; the remainder developed tufts of conidiophores from the beak if kept under moist conditions.

3. Infection experiments indicated the probability of specialised races of the fungus on different hosts, for Sweet William plants were very susceptible while carnations and pinks proved highly resistant.

4. The name *Didymellina Dianthi* is proposed for the new perfect stage, a diagnosis of which is given.

Acknowledgments

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REFERENCES

(1) Berkeley, M. J. *Gard. Chron.* (March 19, 1870), p. 382.
(2) Berkeley, M. J. & Broome, C. E. "Notices of British fungi." *Ann. Mag. nat. Hist.* (1873), pp. 339–49.
(3) Cooke, M. C. "On *Heterosporium*." *Grev.* v (1877), 122–3.
(4) —— *Fungoid Pests of Cultivated Plants* (1906).
(5) Höhnel, F. von. "Mycologische Fragmente." *Ann. mycol.* xvi (1918), 35–174.
(6) Klebahn, H. "Über drei auf Iris gefundene Perithezien und die zugehörigen Konidienpilze." *Ber. dtsch. bot. Ges.* xlii (1924), 60–71.
(7) Lindemuth, H. "Eine neue verheerende Nelkenkrankheit, *Helminthosporium* (*Heterosporium*) *echinulatum*." *Möllers Gärtnerztg*, v (1890). Abst. in *Just's Bot. Jber.* xviii, 2 (1890), 278–9.
(8) Massee, G. *Text Book of Plant Diseases* (1899).
(9) Saccardo, P. A. & Roumeguère, C. *Rev. Mycol.* iii (1881).
(10) Saccardo, P. A. *Sylloge Fungorum*, iv (1886), 481.
(11) Tisdale, W. B. "Iris leaf spot caused by *Didymellina iridis*." *Phytopathology*, x (1920), 148–63.

CORDYCEPS MILITARIS AND *ISARIA FARINOSA*

By T. PETCH

In *Ann. Sci. nat.* Ser. 3, xx (1853), 43, Tulasne stated that he had reason to believe that the conidial stages of *Cordyceps militaris*, *C. entomorrhiza*, etc., were included in the genus *Isaria* as autonomous fungi. Subsequently, in the same journal, Ser. 4, viii (1857), 35-43, he published the results of experiments which, he considered, proved that *Isaria farinosa* was the conidial stage of *Cordyceps militaris*. Figures in support of that conclusion were given in *Selecta Fungorum Carpologia*, iii (1865), Pl. I.

Tulasne obtained a number of dead or dying larvae of *Bombyx rubi*, the victims of a breeding experiment, and placed them on damp sand. They developed a white covering of mycelium, from which arose erect conidiophores, with whorls of branches, each terminated by a chain of conidia. He germinated these conidia in a hanging drop, and found that they reproduced the same conidiophores and conidia. Evidently that was the simple *Spicaria* form of *Isaria farinosa*, though Tulasne described the conidia as spherical, 1·5 μ diameter, and rather confused matters by a reference to *Botrytis Bassiana*. His figures, however, show that he was not dealing with the latter species, and his description of the spores as spherical was perhaps due to the inadequacy of his lenses.

The byssoid covering soon became orange-yellow here and there, and developed *Isaria farinosa*. There is little doubt that Tulasne's identification of that was correct. Subsequently, many of the *Isaria* clavae were attacked by *Melanospora parasitica*.

The experiment was begun in the middle of March. At the beginning of June, some of the larvae, which had produced only a few *Isaria* clavae, or none at all, their segments having remained only more or less whitened by the conidiiferous mycelium, began to develop *Cordyceps militaris*, which matured at the beginning of July. Some of these *Cordyceps* clavae bore conidiophores towards the base.

In *Selecta Fungorum Carpologia*, iii (1865), 6, Tulasne gave further details. He said that the conidiophores and conidia were the same, whether they occurred on the mycelium or on the *Isaria* clavae, and that they were very often to be found also on the "roots" or lowest parts of the *Cordyceps* clava. On germination of the ascospores (part-spores) of the *Cordyceps*, hyphae were produced, from which arose branches bearing chains of conidia or even whorls of chains of conidia.

The conidia produced from the ascospores were exactly similar to those of *Isaria farinosa*.

Tulasne's figures, Pl. I, figs. 19 and 20, show larvae bearing *Isaria farinosa* only. Figs. 21, 22, 23 show germinating conidia of *I. farinosa*, producing whorls of phialides bearing apical chains of conidia, evidently a regular *Spicaria*. Figs. 24, 25, 26 show larvae bearing *Cordyceps militaris* only, not accompanied by *Isaria farinosa*. Fig. 27 is that of the mycelium and conidiophores found at the base of a *Cordyceps* clava, and it shows one, two, or three phialides on the apex of a hypha and others below, but the latter are scattered, not in whorls; only one conidium is present at the apex of a phialide, but the absence of chains might be attributable to loss in manipulation. Fig. 28 is a view of the perithecial clava enlarged. Figs. 29 and 30 show germinating part-spores, and Fig. 31 shows a resultant hypha with conidiophores; the phialides are scattered along the hypha, or three occur on the apex of a short lateral branch; they bear chains of conidia, except in one case where three conidia are shown side by side at the apex of a phialide.

Judging these figures in the light of recent cultures of *Isaria farinosa* and of the ascospores of *Cordyceps militaris*, which will be described later in this paper, it is clear that while Figs. 27 and 31 represent, somewhat imperfectly, the conidial stage of *C. militaris*, they are not figures of *Isaria farinosa*. The fungus shown is not the regular *Spicaria* of *Isaria farinosa*. Other differences will be enumerated in the following pages.

Evidently, as might be expected, Tulasne's material contained a mixture of fungi. Some of the larvae were infected by *Isaria farinosa*, and these produced, first a white mycelium which bore simple *Spicaria* conidiophores, and later the clavae, of *Isaria* (*Spicaria*) *farinosa*. Others were infected with *Cordyceps militaris*, and, as the conidial stage of that fungus is not luxuriant in nature, they bore only a slight covering of conidiiferous mycelium, and ultimately produced *Cordyceps* clavae.

It may be mentioned that, from a collection of pupae of the Cinnabar moth, which had died during a breeding experiment at Farnham Royal, I obtained *Isaria farinosa*, *Beauveria Bassiana*, *Monilia penicillioides*, *Gymnoascus Reesii*, and undetermined species of *Fusarium* and *Stilbella*.

Tulasne's theory met with considerable opposition. De Bary, after carrying out experiments on the subject, at first rejected it, but subsequently accepted it. The following details, taken from de Bary's *Vergleichende Morphologie und Biologie der Pilze*, etc. (1884), give the reason for his change of opinion.

"In [*Bot. Zeitung*, 1869] and 1867, I had expressed doubts concerning the view maintained by Tulasne that *Isaria farinosa* belonged to the cycle of development of *C. militaris*, basing those doubts partly

on the failure to obtain perithecial clavae and *Isaria* forms reciprocally from one another in culture, and partly on differences, certainly only quantitative, in the branching of the conidiophore. The latter objection might easily be dismissed, and, as already remarked in the foregoing text, I now believe that the former cannot be maintained. A caterpillar of *Sphinx euphorbiae*, which had been infected with ascospores and had become, as usual, a sclerotium, when laid on moist sand produced first two small perithecial clavae with normal perithecia. These died before the asci were fully developed, and then *Isaria* was produced in abundance. Portions of the mycelium cultivated on microscope slides had previously afforded *Isaria*. In this case, therefore, either *Isaria* was ultimately produced from the ascospores, or the insect had been infected with *Isaria* unintentionally at the same time as with the ascospores, and the *Isaria* had, in its later development, suppressed and supplanted the perithecial form. I have no reason for assuming such an accidental infection, and have accordingly formed the foregoing conclusion. But the possibility of such an admixture is not excluded, and therefore I could not omit to mention it."

De Bary stated that if the ascospores were sown in water or in a nutrient solution, germination occurred, with branching of the resultant hyphae dependent upon the amount of nutrient present. In water, only short hyphae, with few or no branches, were produced. Some of the branches spread through the nutrient solution as a mycelium; others emerged from the liquid into the air, where they produced whorls of phialides which bore conidia in chains. The first conidium on a phialide was cylindric, like those in the body of the insect, but usually shorter. All the succeeding conidia were globose. The latter could therefore be called globose or aerial conidia. The mycelium which developed in the dead caterpillar very often produced aerial conidia, but no cylindric conidia. The conidiophores which were found on most of the caterpillars which bore perithecial clavae were small, like those described above, and formed a delicate down on the surface. [On the other hand, on other insects they grew into a dense mould-like covering some millimetres in height, or, like the *Coremium* form of *Penicillium*, they formed clavate structures, 1–2 cm. high, with an orange-yellow stalk, covered above with phialides bearing conidia. These last-named bodies were known as a form species under the name of *Isaria farinosa*. Both the *Isaria* form and the mould-covering were usually found alone on the sclerotioid insect, without any perithecial clava.] Only once had he been able to obtain, on a caterpillar which had been infected with ascospores and which pupated after infection, two poorly developed perithecial clavae together with large *Isariae*.

Later in the same account (p. 401) de Bary stated that on insects killed by infection with aerial conidia, perithecial stromata were

never observed, but only a fresh crop of aerial conidia, especially the *Isaria* form.

In the foregoing summary, I have bracketed a section which is obviously a general account of *I. farinosa*, as it occurs in nature, not a series of observations by de Bary from his experiments, and which, as it happens, has no relation to the statements which precede it. Attention may be specially directed to de Bary's statement that the conidiophores which accompanied the *Cordyceps* clavae formed only a delicate down on the surface of the larvae.

De Bary identified as *Isaria farinosa* the conidiophores which he obtained on the germination of the ascospores of *Cordyceps militaris* and those found on caterpillars which bore perithecial clavae of that species. Probably for that reason it is not always clear from his account whether in his infection experiments with "aerial conidia" he made use of conidia obtained by germination of the ascospores or conidia taken from naturally grown specimens of *Isaria farinosa*. For example, in the statement quoted above from p. 401, aerial conidia would appear to mean conidia taken from *I. farinosa*. Nor is it always certain whether his references to "*Isaria*" mean the compound Isarioid form or the individual conidiophores, *e.g.* in his statement that "*Isaria*" developed from mycelium on microscope slides.

It will be noted that de Bary observed differences between the branching of the conidiophores obtained by germination of the ascospores of *Cordyceps militaris* and that of the conidiophores of *Isaria farinosa*, but did not, finally, consider them of importance. Both Tulasne and de Bary appear to have restricted their comparisons to conidiophores obtained by germination of the ascospores of the *Cordyceps* and of the conidia of the *Isaria*, respectively, in their earlier stages in hanging drops. Later, they are quite different. The early conidiophores of the *Isaria* may lack prophialides, and thus resemble to some extent those of the *Cordyceps*, though on the latter, the phialides as a rule are not so regularly arranged in whorls. But the conidiophores which occur on the *Isaria* clava are furnished with whorls of prophialides, each prophialide bearing a cluster of phialides with apical chains of conidia. De Bary's figure, 165 E, as far as regards the branching of the conidiophore, might be matched by the early conidiophores of *I. farinosa* in culture, but the chains of conidia are irregular, and on some of the phialides the conidia are in more or less globose heads, resembling in the latter respect an *Acrostalagmus*.

In 1894, G. F. Atkinson published a paper, "Artificial cultures of an entomogenous fungus," in *Bot. Gaz.* XIX, 129–45, with three plates. From his figures, he was evidently dealing with *Isaria farinosa*, as understood in this country. He did not obtain any perithecial clavae in culture.

In 1895, R. H. Pettit published *Studies in Artificial Cultures of Entomogenous Fungi*, Bull. No. 97, Cornell Univ. Agric. Exp. Sta., Bot. and Ento. Divisions. One of the fungi was *Cordyceps militaris*, cultures of which were made from ascospores (part-spores). The following is taken from Pettit's account:

"Germination from these swollen spore segments takes place by the production of germ tubes at one or two points. These soon become branched....The threads are strongly segmented and the branches are strongly constricted at the base. In some cases a healthy thread suddenly becomes constricted and produces an aborted apex of less than half the diameter of the ordinary thread. The aborted portion is usually curled....In about four days the growth appears above the surface of the agar. A strong white cottony growth appears, forming a colony circular in form. At the end of about six days the conidia appear. Short sterigmata [*i.e.* phialides] are borne near the ends of the long cottony threads. They are irregularly arranged either in an opposite or an alternate manner. They are flask-shaped and slender and sometimes forked. The conidia are nearly spherical and are borne in short chains of three or four at the ends of the sterigmata, or at the end of a long thread. The chains are seldom seen, for they almost invariably collapse, leaving the conidia in balls at the ends of the sterigmata."

Pettit also grew the fungus on potato in tubes. The mycelium on the surface of the potato, and the potato itself, were coloured pale orange or brilliant chrome-yellow, wherever they touched the glass. In a half-litre flask of potato, what was probably the beginning of a perithecial clava, deep reddish orange in colour, was observed at the end of about three months. No *Isaria* forms were obtained.

Pettit's figures show short phialides, narrow flask-shaped, scattered along the hyphae, sometimes opposite, sometimes alternate, sometimes in whorls of three. Two phialides may occur on the apex of a short lateral branch of the main hypha (Pettit's forked sterigma), and a phialide may be produced into a long slender thread. The conidia are shown in short chains, or in a small head at the apex of a phialide.

On comparing these figures with those of Tulasne and de Bary, it is seen that they are essentially the same. De Bary's figure, 165 E, shows apparently a more definite conidiophore, but the phialides are similar, in whorls of three, and the conidia are in heads at the apices of the phialides or in irregular chains. The latter are evidently not the definite chains of a *Spicaria*.

Specimens of *Cordyceps militaris* were collected at Austwick, Yorks., in September 1934. Spore prints were obtained the same night, and

when the specimens were left to dry, the ascospores were extruded in long tendrils which formed fleecy masses over and around the clavae. Cultures were made both from the part-spores in the spore print and from those in the extruded tendrils.

In general, the part-spores germinated readily in hanging drops of water in damp cells. Some of them retained their cylindrical shape. Others assumed an irregular, elongated pentagonal shape, caused by an angular bulging out of one of the longer sides near one end. Some of the part-spores became oval, but these were not observed to germinate.

In the most general method of germination, a short chain of conidia was produced directly from the part-spore, usually from one of the original corners (as seen in profile). Sometimes two chains were produced, sometimes three, from the original corners, or a chain might arise about the middle of the longest side. The first conidium formed was pyriform, with a subacute base, $3 \times 1{\cdot}5\mu$, but the succeeding conidia were subglobose, $2 \times 1{\cdot}5\mu$.

Other part-spores produced a stout hypha, about twice the length of the part-spore, from one end, like a prolongation of the original spore, and then tapered into a conical phialide, which produced a chain of conidia at the apex.

In others, a hypha was similarly produced, but ran through the hanging drop for a considerable length, bearing laterally short, scattered, cylindrical phialides, up to 7μ long, each bearing a chain of conidia at the apex.

Part-spores were also sown on oatmeal agar slants in tubes. Growth was vigorous, and soon the slant bore a thick, greyish white covering, loose and woolly internally, but with an even surface. At the upper edge of the slant, the growth was whiter and somewhat floccose. When old, the covering collapsed into a thin film and became cream-coloured. There was no general growth of erect conidiophores over the surface, nor did it become mealy. The reverse became yellow and then orange, especially where in contact with the glass. No *Isaria* forms have developed in these cultures.

Over the surface of the slant there are no definite conidiophores. Phialides are borne laterally on indefinite hyphae which run in and over the mass of mycelium. These may be alternate, or opposite, or clustered in small groups, sometimes in whorls of three or four, not always at a septum. The phialides are narrow flask-shaped or conical, up to 9μ high, $1{\cdot}5\mu$ diameter below, tapering to the apex. Sometimes a phialide is prolonged into a very fine hypha, up to 50μ or more long.

At the upper edge of the slant, the hyphae more closely resemble conidiophores. They may have a terminal phialide, or a terminal cluster of two or three, with scattered phialides, or whorls of phialides

below. The whorls, however, are often irregular, consisting of about four phialides arising at one level round the hypha, with one or two a short distance above and below. Sometimes a whorl consists of three phialides and a branch, instead of four phialides, as in de Bary, Fig. 165 E, though the branch may be a simple hypha only. There are no prophialides.

The conidia are produced terminally on the phialide. After the formation of the first conidium, it is pushed aside by the growth of its successor. The conidia are strongly mucilaginous, and adhere to one another and to the apex of the phialide, so that a globose head, like that of a *Cephalosporium*, is formed. Alternatively, the conidia may adhere to one another in a single or double chain. In some instances, the mass of conidia arising from one phialide takes the form of a globose head, surmounted by a chain; apparently, in these cases the conidia first formed assumed the chain arrangement, but those formed later grouped themselves round the apex of the phialide.

There is no organic connection between the conidia in a chain, as there is in *Spicaria* and *Penicillium*. They are not truly catenulate, but simply adhere by virtue of their mucilaginous coat. That is very evident when the conidia are mainly oval or subpyriform, as they then adhere to one another with their longer sides in contact, *i.e.* transverse to the direction of production.

The conidia from phialides over the general surface of the slant were chiefly globose, 1·5–2 μ diameter, with some oval, up to 3 × 2 μ, and a few subpyriform. At the upper edge of the slant they were chiefly oval, or subpyriform with one end acute, 2·5–3 × 1·5–2 μ.

De Bary noted that the first conidium produced by a phialide was cylindric, like the cylindric conidia found in the body of the insect but shorter. That phenomenon occurs in culture, but apparently not universally. One would not, however, call this first conidium cylindric. It is rather pyriform, with a truncate base, up to 6 μ long and 3 μ diameter. Frequently this large conidium is perched on the top of a globose cluster of conidia (compare de Bary, Fig. 165 E), or at the apex of a chain of conidia. More peculiar is the fact that in the latter case, when the conidia in the chain are oval and stand transverse to the direction of production, the large terminal conidium stands in the direction of production and consequently at right angles to them.

The results detailed above are in agreement with those obtained by Tulasne, de Bary, and Pettit from cultures of the ascospores of *Cordyceps militaris*. They also agree with those obtained by Mr E. W. Mason, of the Imperial Mycological Institute, in unpublished research on the same subject. The conidial stage of *C. militaris* is a Mucedine, with phialides arranged laterally on indefinite hyphae, though towards the ends of the hyphae they may be grouped so as to simulate

a branched conidiophore. The conidia are borne terminally on the phialide and usually adhere in a globose head. The fungus is perhaps best regarded as a *Cephalosporium*. It is certainly not *Spicaria* (*Isaria*) *farinosa*, which has regular whorls of prophialides and broader flask-shaped phialides, with truly catenulate conidia.

Tulasne and de Bary were mistaken in identifying the conidial stage of *Cordyceps militaris* with *Isaria farinosa*. In other respects their morphological results agree with those of subsequent workers. The only detail which conflicts with this conclusion is de Bary's experiment in which the perithecial clavae of *Cordyceps militaris* and large Isariae developed from the same pupa. With regard to that there are two possible explanations. The first is that the larva was infected by *Isaria farinosa*, before de Bary infected it with ascospores of the *Cordyceps*. The second is that the conidial stage of *C. militaris* may, under some conditions, assume an Isarioid shape, just as *Isaria farinosa* may occur as a simple *Spicaria* or as an *Isaria*. But as such an Isarioid form has not occurred in culture, nor been found on a pupa in nature, this second explanation would appear improbable.

As noted by Tulasne and de Bary, the conidiophores of *Cordyceps militaris* may occur on the mycelium on the larva or pupa which bears the perithecial clavae. Apparently they are not always present, or rather, I have failed to find them sometimes. Those I have seen are conical phialides, up to 12 μ high, 1–1·5 μ diameter at the base, tapering to the apex. They are lateral on the hyphae, or two or three are situated at the apex of a short lateral branch. In one instance, a group of three phialides was seen on the apex of a hypha. The conidia adhere in short chains, or in a small cluster, at the apex of a phialide, and are globose, 1·5 μ diameter, or oval, 2 × 1·5 μ.

During the last five years, I have made numerous cultures of *Isaria farinosa* with the object of ascertaining whether any specific differences could be established between the different forms of that species, or between the examples which occurred on different hosts. Apart from the fact that *Isaria farinosa* has a definite *Spicaria* conidiophore, cultures of it differ in appearance from those of the conidial stage of *Cordyceps militaris*. They are much looser and more woolly at first, and soon become covered with conidiophores and conidia which give them a mealy appearance. Ultimately, on oatmeal agar or similar media, they produce *Isaria* clavae.

Isaria farinosa has been identified on Lepidopterous larvae and pupae, Hymenoptera, Coleoptera, Aphides, Diptera, and Arachnida. On the larger of these it forms conidial clavae, but on the smaller, and sometimes even on the larger, it produces only a covering of conidiophores. Now that its association with *Cordyceps militaris* has been terminated, one has less difficulty in accepting the identity of the fungi on these different hosts. It is evidently an omnivorous

entomogenous fungus which can attack insects of all kinds. On Hymenoptera, it has been named *Coremium Swantonii* A.L.Sm.

Summary

The conidial stage of *Cordyceps militaris* is a *Cephalosporium*, which occurs on the mycelium on the larva or pupa bearing the *Cordyceps*. It has not been known to produce an *Isaria* form in nature or in culture.

Isaria farinosa is a fasciculate *Spicaria*, and may occur as an *Isaria* or as a simple *Spicaria*. Its perithecial stage, if any, is unknown. It has no relation to *Cordyceps militaris*.

Cordyceps militaris attacks the larvae and pupae of Lepidoptera. There are two records of its occurrence on Coleoptera—on the remains of a cockchafer in the wood of Bailly, Aube, France (Briard), and on a cockchafer, Aude, France (Roumeguère)—but these are generally considered to be erroneous.

Isaria farinosa is a general entomophyte, and is known to occur on Lepidoptera, Hymenoptera, Coleoptera, Diptera, Aphides, and Arachnida.

Cordyceps militaris of the United States of America is the same as the European species.

SPORES AND SPORE GERMINATION IN WILD AND CULTIVATED MUSHROOMS (*PSALLIOTA* SPP.)

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(With Plate III and 2 Text-figures)

Hitherto the classification of the various forms of field and cultivated mushrooms has been based on external morphological characters only, and no account has been taken of the number of spores on the basidium. It has been known for some time that the typical wild species of *Psalliota campestris* and *P. arvensis* have four-spored basidia, with occasional exceptional two-spored individual caps. Sachs in 1868 (see English edition, 1882, Fig. 227) figures two-spored basidia of *Agaricus campestris*, and Buller (1909) records finding two-spored individuals in the wild on manured ground in the campus of the University of Manitoba, but he states that "they differed considerably from the wild field mushrooms of England, in that they were more scaly, browner and possessed relatively very shallow gills". Buller's specimens were evidently not typical *Psalliota campestris*, and unfortunately the origin of Sach's specimen is not known. If two-spored forms of typical *P. campestris* occur in the wild they are rare, and are possibly haploid fruiting bodies of the four-spored type.

It has been stated by Buller and others that the cultivated varieties have two-spored basidia. It is true that two-spored basidia predominate, but, on examination, only one out of three cultivated varieties on the market in this country has shown uniformly two-spored basidia; in the others an appreciable percentage of three-spored basidia occur together with a few one- and four-spored. The variable basidia are not evenly distributed over the gills but are found in patches; Pl. III shows two photomicrographs of the young gills of the fuscous (fig. 5) and the white non-fragrant (fig. 4) cultivated varieties. None of the cultivated varieties examined has shown uniformly four-spored basidia as in the wild species.

The three different varieties of cultivated mushrooms examined and tested for spore germination are as follows:

(1) The fuscous variety on the English market; coarse, thick fleshed, with thick bulbous stipe, tough and tasteless when cooked; basidia and spores variable, basidia one- to four-spored (Pl. III, figs. 1, 1 *a*).

(2) The white non-fragrant variety, also on the market, softer in texture, flesh thinner than in (1), closely resembling the wild *P. campestris*; slight but delicate flavour when cooked; basidia and spores variable, basidia one- to five-spored (Pl. III, figs. 2, 2 *a*).

(3) The white fragrant variety, closely resembling *P. campestris* but rather drier and firmer in texture, with a strong smell when fresh; good texture and flavour when cooked; basidia uniformly two-spored.

In an appendix at the end of this paper full technical descriptions of (1) and (2) are given by Miss E. M. Wakefield. Unfortunately the white fragrant variety (3) has been met with only once; it was bought with stipe cut short, and it has not been possible to procure any more perfect specimens. A full technical description of this and other forms met with under cultivation will therefore have to be deferred to a later publication.

Nothing is definitely known as to the origin of these cultivated forms. The Americans believe that the coarse fuscous variety, which they call *P. brunnescens*, originated in this country and was selected presumably from spawn gathered in the wild; but the wild species *P. brunnescens* is not common either in this country or in the States, and there is no record of its being introduced into cultivation.

There are, however, three records of a form very similar to our fuscous variety occurring in the States. Atkinson (1906) obtained material for his investigation on the development of *Agaricus campestris* from cultures grown in a greenhouse of a two-spored variety of *A. campestris* known as "Columbia", sold by the Pure Culture Spawn Co. of Missouri. This variety is figured and closely resembles the fuscous variety described by Miss Wakefield in the appendix. The stipes of mature pilei of "Columbia" as figured by Atkinson are, however, not bulbous. This may only be due to cultural conditions as the immature unexpanded specimens clearly show the typical bulbous stipe.

Atkinson states that he twice found two-spored *Agaricus* closely resembling certain cultivated forms growing spontaneously in the open; on a lawn which had been mulched with horse manure, and on a hill side of a wooded ravine in the campus of Cornell University.

Murrill (1914) found a number of caps of a fuscous *Psalliota* on an old heap of manure in Bronx Park, New York. He described and figured it in *Mycologia* (1914), and gave it the name of *Agaricus campestre hortensis*. His photographs are not quite clear, and his specimens appear to differ slightly from the common fuscous form on our markets. They are, however, not at all unlike.

F. C. Stewart (1929) purchased spawn from an American Spawn Co., alleged to be the "cream white" variety, but all the pilei produced by this spawn were, as he says, "altogether different from the common mushroom", and, judging from his figures and description,

there is no doubt that he was dealing with the fuscous variety common in this country. Stewart sent specimens to Dr Kauffmann who, after careful examination, expressed the opinion that they might belong to *P. brunnescens*, a species described by Peck (1929), and that he knew of no other species to which the specimens approached more closely; and further that this fuscous form should not be considered as a variety of *P. campestris*, *P. arvensis* or *P. subrufescens*. Murrill also thought that his form seemed very near *P. brunnescens*, and states that it is often cultivated but rarely wild. Peck's figures and description, however, differ in several respects from the fuscous form figured and described by Stewart.

There is also nothing definitely known about the origin of the two white cultivated forms, the fragrant and non-fragrant, but they must have originated at some time from wild species.

The two-spored cultivated varieties are not haploid forms of the four-spored species. The young basidia are binucleate, karyogamy occurs in the basidium, followed by two meiotic divisions resulting in four daughter nuclei, which can be seen distinctly in a resting state just as the sterigmata are about to develop. Sass (1928) describes the passage of two nuclei into each spore in the two-spored form he investigated, and Colson (1935) the same for the two-spored form, and one nucleus into each spore in the four-spored. The nuclei divide again once in the young spore.

Spores

A number of spores of *Psalliota campestris*, *P. arvensis*, and the several varieties of cultivated mushrooms have been stained and examined for nuclei.

The technique is as follows:

A small platinum loopful of egg albumen, such as is used for mounting microtome sections, is placed on a clean grease-free slide, and spores from a dry spore-trace placed in the drop. The drop is then spread about 1 in. along the slide with a clean glass rod, to ensure the film being thin and even. The slide is then allowed to dry partially for twenty-four hours, protected from dust.

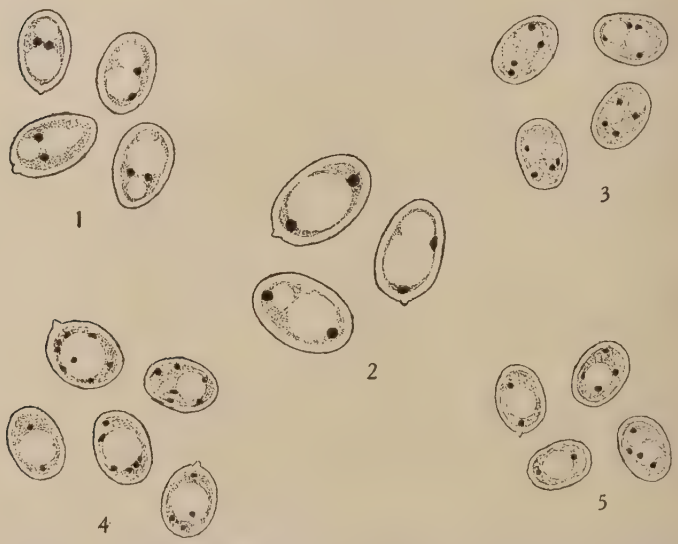
Then the slide is fixed in a fixative recommended by Sass (1929):

1 per cent. glacial acetic acid	40 c.c.
Commercial formaldehyde...	10 c.c.
95 per cent. alcohol...	50 c.c.

for twelve to twenty-four hours, rinsed thoroughly in 50 per cent. alcohol and taken down to water. This fixative hardens the film and the spores do not wash off.

The spore walls of *Psalliota* are dark coloured and somewhat thick, more especially in *P. arvensis*. For the decolorisation of the spore walls

a modification of the method recommended by Brunswik (1924) should be used. Brunswik differentiated with Eau de Javelle after staining with Heidenhain's haematoxylin, but it was found that clearer and better preparations resulted if the process is reversed and the spore walls decolorised before mordanting and staining. The preparations should be left in the Eau de Javelle for half to one hour or longer, until the walls are completely decolorised, then washed thoroughly in water, mordanted in 3 per cent. iron alum for three to four hours, stained overnight in 0·5 per cent. haematoxylin and



Text-fig. 1. 1, wild *Psalliota campestris*; 2, wild *Psalliota arvensis*; 3, cultivated white fragrant form; 4, cultivated white non-fragrant form; 5, cultivated fuscous form. ×2150.

differentiated in the usual way in 2 per cent. iron alum, until nothing but the colourless spore outlines can be seen under the low power of the microscope. Differentiation requires a little practice, as the nuclei are not visible under a low magnification.

The results obtained from the examination of these spores confirm Sass's observations (1928) on the two-spored form he worked with, and Colson's results recently published (1935), with two- and four-spored forms of *Psalliota*, namely that spores from two-spored basidia are quadri-nucleate (Text-fig. 1 (3)) and those from four-spored basidia binucleate (Text-fig. 1 (1), (2)).

The spores of the fuscous cultivated form with variable basidia (one- to four-spored), showed both binucleate and quadrinucleate spores in the same spore-trace (Text-fig. 1 (5)). The white non-fragrant cultivated form, also with variable basidia, but mainly two-spored, showed spores with from two to eight nuclei (Text-fig. 1 (4)).

Rough counts made in microscope fields taken at random on young gills of the fuscous and non-fragrant white forms are given in Table I.

Table I. *Number of spores on basidia in cultivated forms*

Pileus	Basidia counted	Percentage				
		1-spored	2-spored	3-spored	4-spored	5-spored
		White non-fragrant form				
I	743	4·8	88	6·6	0·5	—
II	359	10	85	5	—	—
Total	1102	6·7	87·2	5·6	0·36	—
		Fuscous form				
I	250	6·8	37·6	48·4	7·2	—
II	250	1·2	49·2	42·8	6·8	—
III	950	3·4	62·6	29·1	4·4	0·3
Total	1450	3·6	56	34·8	5·3	0·2

Spore measurements

Wild species. Basidia four-spored:

Psalliota arvensis ... ... 8·9–11·1 × 6·4–7 μ
P. campestris ... ... 7·6– 8·9 × 5·1–6·4 μ

Cultivated varieties. Basidia two-spored or variable:

Fuscous ... ... 5·7– 8·9 × 5·1–7 μ
White non-fragrant ... ... 6·4– 8·9 × 5·1–7 μ
White fragrant (two-spored) ... 6·4– 7·6 × 5·1–5·7 μ

Spore germination

An enormous amount of work has been done by various investigators on the conditions conducive to the germination of spores of *Psalliota*. A good review of the literature up to 1924 can be found in Falck's paper (1924) and need not be gone into *in extenso* here.

Hoffmann (1860) is probably the first investigator who succeeded in germinating spores under controlled conditions, in water or damp air. He does not appear to have had any difficulty and remarks that there is nothing unusual about the germination of *Agaricus campestris*.

Attempts at mushroom culture under controlled conditions were first started in France. Chevreul (1861) and others tried test-tube cultures with very variable and uncertain results.

In America Duggar (1901) and Ferguson (1902) were the first to attempt to germinate the spores of various fungi including *A. campestris*, on media of known constitution, previously subjecting them to various treatments including artificial digestive fluids. Duggar, in his paper, employed the generic name *Agaricus* in the sense in which it is usually understood "by those interested in the practical side of the work", and gives no detailed description of the forms or varieties he worked with. This was of course before the days of pure spawn culture.

Both these investigators had very variable results. Ferguson, however, made the important discovery that germination could be stimulated by the introduction of small pieces of vigorous mycelium from a culture of the same species into the spore suspensions. She obtained her spores from sporophores just as the veil was about to break and again when the pileus was fully expanded or nearly so. She observed that spores from one spore-trace from a fully expanded sporophore raised in a conservatory, gave more uniform germination than those from any other spore-trace, but gives no further details as to the origin of the other prints, whether from cultivated forms or from the wild. She also noticed that when a high percentage of germination finally resulted it was always preceded a few days before by the germination of one or more spores. She was wholly unable to account for the irregularities in her results, but suggests that the maturity or other conditions of the spores must be the cause.

In 1924 Falck made a series of very elaborate experiments on the germination of spores of *Psalliota*, treating them with organic and inorganic acids, alkalis, etc., and obtained the best results in a mixture of 0·25 per cent. succinic acid and 10 per cent. malt extract. He repeated Ferguson's experiments and found that germination was stimulated by the introduction of mycelium of the same species. But, again, he gives no definite description of the form he dealt with.

After Falck, little further work appears to have been done on germination until Lambert in 1929 succeeded in isolating single spores of a cultivated variety "snow white", and in obtaining sporophores from single spore cultures, and from all possible combinations of them.

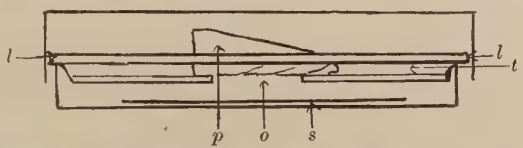
In 1930 Hein was able to germinate spores in distilled water, dung decoction and various synthetic media. The spores were obtained from mushrooms grown for experimental purposes in a specially constructed house. He gives no description of the variety used, but in all probability he was dealing with one or other of the cultivated forms.

The main object of this paper is to describe a method for spore collection and a simpler and fairly reliable method for inducing germination in the cultivated varieties but more especially in the wild

species *P. campestris*. The spores of the cultivated varieties germinate more readily than those of the wild species.

Without previous treatment, the spores require an incubation period of from six to seven days before they germinate, even under the best conditions; and, in order to avoid contaminations, the less they are handled before being placed in the solution in which they are to germinate the better.

Previous preliminary experiments on spore germination in Knop solution only, showed that the condition of the sporophore when the spore-trace is taken is of prime importance. The first shed spores of an immature sporophore do not germinate under artificial conditions. The pileus must be fully expanded and the gills umber, and consecutive traces should be taken from the same pileus at intervals of twelve to twenty-four hours. Therefore, it is advisable when gathering mushrooms in the open, not to gather the cap only, but to cut out the sod on which the cap is growing, so that it can continue to develop fairly normally for some days. The sod can either be left out



Text-fig. 2. Diagram of Petri dish fitted with tin tray and slide, and with sector of pileus in position for collecting spore trace. *l*, lip of tray resting on the edge of lower half of Petri dish; *o*, opening in the tray; *p*, sector of pileus; *s*, slide; *t*, tray.

of doors in a sheltered place, or brought into the laboratory and placed under a bell jar, which should be slightly tilted to ensure adequate ventilation without risk of drying out. If it is not possible to cut out the sod, the stipe should be pulled up gently and not cut. The pileus can be kept in a fresh condition by placing the base of the stipe on damp cotton wool in a small tube covered with a bell jar, again giving adequate ventilation. Sectors of the pileus can then be cut out when required.

The following is a method of collecting spore-traces free from contamination:

The quarter pound squat tins of Player's "Country Life" tobacco have lids which fit into a 4-in. Petri dish, so that the lip of the lid rests on the edge of the lower half of the dish, leaving a space of about $\frac{1}{4}$ in. between the lid and the base of the dish. The decoration can be easily removed with methylated spirit and a rectangle cut out of the centre of the lid. A sterile slide is then placed in a sterile Petri dish and the tin tray flamed and placed over the slide. A sector is then cut out of the pileus and placed over the slit in the tray and the lid of the dish replaced (Text-fig. 2). Sectors of large pilei do not

allow of the lid of the dish fitting down closely, but as long as the sector is large enough to cover the slit completely there is no risk of outside contamination of the trace. The slide with the spore-trace can be removed after the required interval and replaced by another, and a fresh sector of the pileus placed on the tray, and so on, until spore discharge ceases.

Before storing, the slides should be allowed to dry for twenty-four hours, then another sterile slide placed over the spore-trace and the two slides fixed together at one end with a sticking label, leaving the other free for opening when required. The slides should be wrapped tightly together in cellophane or clean paper, and stored in a dry cool place.

The spores of *P. campestris* remain viable for about six months, those of the cultivated varieties rather longer. Spores of the fragrant white variety have germinated after eight months and one spore-trace of the fuscous form gave some germination after fifteen months.

Method for germinating the spores

A hollow-ground slide is placed in a Petri dish on a circular piece of filter paper with an oblong cut out of the centre to coincide with the hollow in the slide. This eliminates the necessity of removing the slide from the dish for examination under the microscope. The dish is then sterilised. When sterile, a few drops of Knop solution are pipetted into the hollow of the slide, and a loopful of spores from a trace gently dipped into the liquid and not stirred, so that a number of spores remain floating on the surface. Although germination generally begins amongst the submerged spores, the floating spores appear to do better and grow faster.

The filter paper is then moistened with sterile water, the dishes placed on a sheet of glass under a bell jar lined with damp blotting paper and left to incubate undisturbed for about seven days at a temperature of 26–27° C. The Petri dishes should be marked with a wax pencil and not with sticking labels, as *Penicillium* and other impurities develop on the paper in a saturated atmosphere and considerably increase the risk of contamination when the dishes are opened.

Small pieces of vigorous mycelium may be placed at the edge of the Knop solution either at the time of sowing or seven to ten days after. The latter method was found to be the better in practice.

The objections to inserting the inoculum at sowing are that during the incubation period of seven to ten days the mycelium spreads all over the surface of the Knop solution and may absorb most of it, and also, when spores from a trace are capable of germinating without any inoculum, germination may be delayed for a few days.

If the inoculum is introduced after ten days, germination often starts within twenty-four to forty-eight hours and is much more even. The stimulus set up by the inoculum hardly extends beyond the tips of the hyphae, but once a few spores have germinated, a secondary stimulus is set up and germination spreads throughout the liquid.

If the sporelings are required for growing on, they should be taken out of the Knop solution as soon as possible and spread on a solid or semi-solid medium, from which they can be picked off singly or in groups and transferred to slopes of oatmeal agar.

Spore germination tests

Table II gives two series of germination tests with spores of the wild species *Psalliota campestris*, in Knop solution, with and without inocula from stipe cultures of the same species.

LS 7, LS 7*a*, and LS 7*b* are three consecutive spore-traces from a mature pileus with umber gills, taken at the intervals stated. It will be seen that in Knop solution only, without any inoculum, the spores of the first spore trace (LS 7) gave the best results on the eleventh day in series A, and some germination on the eighth day in series B. The third trace (LS 7*b*) gave little or no germination in B until the seventeenth day.

With the addition of inoculum at sowing, the second trace proved the best on the tenth day in series A, and gave some germination on the eighth day in series B. With the addition of inoculum on the tenth day the third trace (LS 7*b*) gave good germination in twenty-four hours in series A, and in B a percentage equal to that of LS 7 in series A.

The Knop solution in both series was not freshly made, but had been kept in tubes for some weeks before use.

It must be pointed out that these percentages were obtained from counts in microscope fields taken at random in the region within the influence of the stimulus set up by the inoculum, either underneath or round the edge of the mycelium. They are consequently merely rough estimations and only serve as a means of comparison between the effects produced by the different treatments. A true estimate of the percentages would entail spreading the spores from the suspensions daily on a solid or semi-solid medium for several days in succession, allowing them to incubate and counting them. Neither time nor incubator space would allow of this method being adopted with a large number of spore-traces; but, in one single instance, the percentage of germination was considerably increased by plating and counting the spores some days after germination had started in the suspensions.

Psalliota does not do well in a solution. After the germ tubes have

Table II A. *Germination tests in Knop solution.*

Spores 7 weeks 3 days old

Date ...	10th day			11th day			14th day		
Spore-trace, *P. campestris*	No. inoculum	Inoculum added at sowing	Inoculum added after 10 days	No inoculum	Inoculum added at sowing	Inoculum added after 10 days	No. inoculum	Inoculum added at sowing	Inoculum added after 10 days
				P. campestris with *P. campestris* inoculum. Medium Knop.					
LS 7, gills umber 1st 16 hr.	–	+ 31	–	++ 45·7 %	+	Occasional spore	++	++ 46·8 %	A little
LS 7*a*, 16–40½ hr.	–	++ 53 %	–	–	++ 59 %	Occasional spore	A little in patches	++	+ In patches
LS 7*b*, 40½–64 hr.	–	+	Occasional spore	–	++ 50·6 %	++ 48·4 %	–	++	++

Table II B. *Spore germination tests in Knop solution.*

Spores 8 weeks 6 days old

Date ...	7th day		8th day		10th day		17th day	
Spore-trace	No inoculum	Inoculum added at sowing	No inoculum	Inoculum added at sowing	No inoculum	Inoculum added at sowing	Inoculum added after 10 days	Inoculum added at sowing
			P. campestris with *P. campestris* inoculum. Medium Knop.					
LS 7	–	+ Occasional spore	+ Occasional spore	+	Occasional spore + inoculum added	–	56 %	25 %
LS 7*a*	–	–	–	A little + in patches	–	Nothing further	34·2 %	50 %
LS 7*b*	–	–	–	–	– Inoculum added	–	27 %	59·5 %

attained a certain length, the spores are either pulled up to the surface of the liquid and there get caught up in the inoculum, or, if they remain submerged, the germ tubes die and disappear. If the inoculum is very vigorous, it is often necessary to draw it aside to expose the spores lying underneath, and a certain number of sporelings are drawn away with it. This mainly accounts for the decrease in the percentages in series B after the twelfth day. Also after the germ tubes of the floating spores have attained a considerable length they become so entangled that they cannot be counted.

The patchy germination recorded in this table is most probably due to a secondary stimulus set up by the scattered germinating spores seen on the eleventh day and not to the stimulus set up by the inoculum.

Table III gives a more comprehensive series of germination tests, with inocula of the same and different species. The same spore-traces LS 7, LS 7*a*, LS 7*b* as in Table II are included, when the spores were four months old; also two spore-traces from another pileus of *P. campestris* S 32, S 32*a*, gathered when the gills were not fully ripe but pinkish umber in colour. CP 4*a* is the second spore-trace of the white fragrant cultivated variety.

Table III. *Germination tests with inocula of the same species and different species*

		Same species *P. campestris*		Different species *P. arvensis* cultivated	
Spore-trace *P. campestris*	No inoculum	Inoculum same species added at sowing	Inoculum same species added after 7 days	Inoculum *P. arvensis* after 7 days	Inoculum fuscous form after 7 days
		Date: 10th day after sowing			
		Fresh Knop 23. ii. 35			
LS 7, + 1st 16 hr. gills umber	+	+	A few spores	+ Occasional spore	+ 36·3 %
LS 7*a*, + 16–40½ hr.	+ Occasional spore	−	+	+ Occasional spore	+ 32·9 %
LS 7*b*, + 40½–60 hr.	+	−	+	+ Occasional spore	+ Occasional spore
S 32, 1st 16 hr. gills pinkish umber	+ 1 spore	+ 1 spore	−	+ Occasional spore	+ Crystals; a little germ.
S 32*a*, 16–29 hr.	−	+ A few spores	+ A little germ.	−	+ Crystals; fair germ.
		White fragrant cultivated variety			
CP 4*a*, 17–37½ hr. gills umber	Contaminated with bacteria	+	+ 43 %	A few spores	+

Table III (*continued*)

Spore-trace *P. campestris*	No inoculum	Same species *P. campestris*: Inoculum same species added at sowing	Same species *P. campestris*: Inoculum same species added after 7 days	Different species *P. arvensis* cultivated: Inoculum *P. arvensis* after 7 days	Different species *P. arvensis* cultivated: Inoculum fuscous form after 7 days
		Date: 12th day after sowing			
		Fresh Knop 23. ii. 35			
LS 7, + 1st 16 hr. gills umber	+	+	+	+	++ 50·8 %
LS 7*a*, + 16–40½ hr.	Occasional spore	–	+	Occasional spore	++ 52·8 %
LS 7*b*, + 40½–60 hr.	+	+ Occasional spore	+	+ Occasional spore	++ 28 %
S 32, 1st 16 hr. gills pinkish umber	+	Nothing further	+	Occasional spore	+ Crystals; nothing further
S 32*a*, 16–29 hr.	+	+ Occasional spore	+	–	+ Crystals; nothing further
		White fragrant cultivated variety			
CP 4*a*, 17–37½ hr. gills umber	– Bacteria	Crystals; nothing further	++ 43·1 %	Early ++ stages 44·9 %	++ 40·0 %
		Date: 16th day after sowing			
		Fresh Knop 23. ii. 35			
LS 7, + 1st 16 hr. gills umber	+ Early stages 17·7 %	++ Early stages 30·2 %	+ Nothing further low %	+ 11·2 %	++ 48·8 %
LS 7*a*, + 16–40½ hr.	+	+	+ Not countable	+ Nothing further	++ 35 %
LS 7*b*, + 40½–60 hr.	+ Low %	+ Nothing further	+ 21·9 %	++ 26 %	++ 28·5 %
S 32, 1st 16 hr. gills pinkish umber	+ Nothing further	+ Nothing further	+ Early stages 12 %	+ Nothing further	+ Crystals; nothing further
S 32*a*, 16–29 hr.	+ Nothing further	+ Nothing further	+ Nothing further	+	+ Not countable
		White fragrant cultivated variety			
CP 4*a*, 17–37½ hr. gills umber	Occasional spore; bacteria	+ Nothing further	++ Floating spores 33·4 %	++ 36·8 %	++ 41 %

LS 7, LS 7*a*, LS 7*b*, spores 4 months old.
S 32, S 32*a*, spores 4 months 10 days old.
CP 4*a*, spores 8 months 18 days old.

It will be seen that all the three traces of LS 7, although older than in Table II, have shown some germination on the tenth day in Knop solution only, but germination has been retarded in the spore suspensions of LS 7*a* and LS 7*b* by the inoculum introduced at sowing, and this retardation continues until the sixteenth day.

The inocula of *P. arvensis* introduced on the seventh day do not appear to produce any marked stimulatory effect, and the percentage recorded on the sixteenth day may be attributed mainly to the secondary stimulus set up by the presence of some germinating spores as in the controls without inoculum.

The stimulatory effect of inocula of the fuscous cultivated form, on the other hand, is very marked on the third day after the introduction of the inoculum, in the first two spore-traces of LS 7, and still more marked on the fifth day. The third trace LS 7*b* does not respond so readily, and the effect is not more pronounced than with inocula of *P. campestris* itself. In this series the first two traces have given the best results.

With regard to S 32 and S 32*a* the spores from pinkish umber gills, the percentages of germination are low throughout. The crystals recorded in the suspensions with the fuscous inocula require some explanation, as they produce an inhibiting effect which has to be taken into account. The aerial mycelium of both wild and cultivated forms of *Psalliota* is thickly encrusted with crystals, analysed by Hein (1930) and found to be calcium oxalate. The fuscous form produces a considerable amount of fluffy aerial mycelium on oatmeal agar. Some of this aerial mycelium was introduced in the inoculum, with the result that crystals were deposited in the Knop solution. These crystals dissolve slowly and completely alter the chemical constitution of the Knop solution and further germination is inhibited.

The effect produced by the different inocula on the fragrant white cultivated form CP 4*a* is interesting. The fuscous inoculum is not more potent than the inoculum of CP 4 itself, although they are both cultivated forms. Again, the suspensions containing crystals show inhibition of growth and further germination, and accidental contamination with bacteria is also deleterious. In other germination tests with CP 4*a*, this trace has shown that the spores are capable of germinating in Knop solution only.

Besides the tests recorded in Tables II and III, many other tests with spores of *P. campestris*, with and without inocula, have shown that success mainly depends upon the maturity of the sporophore when the traces are taken. During spore discharge from one and the same pileus, there appears to be an optimum germination period, either without inocula, or with certain varieties of inocula, and that this period can be shifted by subjecting the spores to other stimuli. Hence the advisability of taking consecutive spore-traces.

During this investigation most of the spore traces obtained by the method described on p. 231 have been free from contamination. Provided fresh, clean pilei are selected and due care exercised, the risk of contamination of spore traces from fully expanded pilei is not great.

Attempts at germinating spores of *P. arvensis* have failed. This is probably due to the lack of spores from mature sporophores, as the spores obtained at the beginning of this investigation were all from immature pilei, and the following autumn no further material was found.

Discussion

In view of the uncertainty about the origin of the cultivated varieties dealt with in this paper, no object is to be gained, at this juncture, by classing them as varieties of any one or other of the wild species *Psalliota campestris*, *P. arvensis*, *P. brunnescens*, etc., until proof is forthcoming whether they are or are not mere varieties. They are therefore described merely as cultivated forms of *Psalliota*. With the exception of the fuscous form, the other white cultivated forms more nearly approach *P. campestris*, than any other wild species met with so far.

It has been pointed out above that, apart from morphological characters, the main difference between the cultivated forms and the wild species is the number of spores on the basidium, and the number of nuclei in the mature spores. Lambert has shown that the spores of the cultivated "cream white" variety, with two-spored basidia, is monoecious; and since each spore receives two basidial nuclei, the spores are presumably in the diplophase. So far, there is no record of heterothallism in the genus *Psalliota*, but the fact that, in the four-spored wild species the spores receive only one instead of two basidial nuclei, and must consequently be definitely haploid, suggests that *P. campestris* and *P. arvensis* may be haplo-dioecious. Another possible difference between the wild and cultivated mushrooms may be that the cultivated forms can complete their life history from spore to spore as saprophytes, whereas there is some experimental evidence, not yet published, that a form of symbiosis exists between various grasses and the field mushroom *P. campestris*. Artificial inoculations under sterile conditions have shown that the mycelium can penetrate and live in the roots of grasses for a time without materially affecting the growth of the grass plants. It is not known how long this symbiotic balance persists; but, judging from the examination of roots from sods carrying pilei gathered in the open, the infected roots eventually die and are replaced by new growth in the root system. In the open, however, the grass evidently derives some benefit from the presence of the spawn of *P. campestris*,

as can be seen on fairy rings formed by this species. The grass on the rings is deeper green and of a more luxuriant growth than either inside or outside the ring. The mycelium of *P. arvensis* will also penetrate and live in grass roots, but in the open, the spawn is so profuse that it sets up physical and possibly chemical conditions, such as impermeability to water and excessive deposits of calcium oxalate, which are detrimental to the deeper rooted grasses.

Summary

The basidia of the two wild species *Psalliota campestris* and *P. arvensis* and three different forms of cultivated mushrooms vary in the number of spores on the basidium. The wild species have four-spored basidia and the cultivated either two-spored or from one- to four-spored.

Only one out of these three cultivated varieties has shown uniformly two-spored basidia.

The number of nuclei in the mature spores of the wild species is constant; they contain two nuclei. In the cultivated variable varieties the number of nuclei may range from two to eight.

Methods are given for obtaining consecutive spore-traces from single pilei, for staining spores for nuclei, and for spore germination.

The stage of maturity of the sporophore when the spore-traces are taken is all important.

In conclusion I wish to acknowledge my indebtedness to Miss E. M. Wakefield for her help and for the technical descriptions at the end of this paper; to Mr H. G. Osterstock for the photomicrographs; to the laboratory assistant A. F. Emarton for the photographs reproduced in Pl. III; and to the foreman Mr J. Newell for specimens of the fuscous and non-fragrant white cultivated varieties.

APPENDIX

DESCRIPTION OF TWO FORMS OF CULTIVATED MUSHROOM

By E. M. WAKEFIELD

White form

Pileus up to 7 cm. in diameter, at first hemispherical, then flattened-convex, margin incurved and extending beyond the gills.

Surface of pileus smooth, soft to the touch like a kid glove, under the lens appearing made up of silky fibrils, pure white, but becoming stained with brown when handled.

Flesh 12–13 mm. thick in the centre, attenuated towards the margin, white, solid, but less firm than in the brown form, becoming slightly pinkish when cut.

Lamellae crowded, reaching the stem but free from it, rounded behind, narrow, thin, at first pale, clear flesh-pink, becoming purplish brown (warm sepia (*R*)) as the spores mature.

Stipe 6–8 cm. long by 1½–2 cm. broad, white, tinged rosy purple at the apex, smooth with a satiny sheen, equal or slightly incrassated at the base, becoming rufescent when rubbed.

Veil white, membranous, silky, thin and very frail, when ruptured leaving an irregular annulus on the stem.

Annulus median, white, at first broad and flaring upwards, but soon collapsing and eventually almost disappearing.

Basidia two-spored. Spores broadly elliptical, very variable in size, and also variable in colour, some being quite pale while others are more or less deep reddish brown.

Spore print dark brown (sepia (*R*)).

Spores 6·4–8·9 × 5·1–7 μ.

Brown form

Pileus up to 9 cm. broad, convex (flattened and hemispherical) when young, with margin strongly inrolled, becoming convex-expanded with age, with a slight depression in the centre.

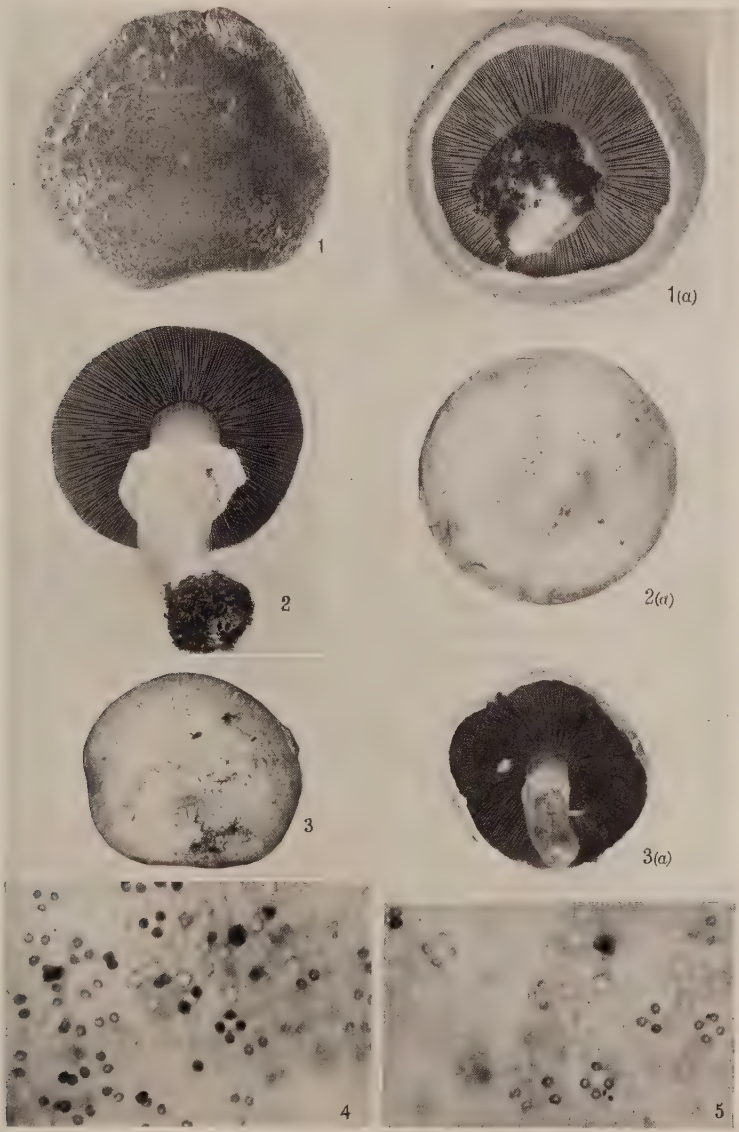
Surface of pileus in the unopened state somewhat tomentose, avellaneous (*R*) in colour with sometimes, especially towards the disk, slightly broken up into scales. As the pileus expands the scales become more evident, and especially on handling or on drying the colour becomes deeper brown and the scales appear fibrillose and closer adpressed, especially towards the margin. Margin whitish and exceeding the gills, in expanded specimen often recurved, striate rim consisting of fragments of the veil.

Flesh very thick, compact, firm, whitish or tinged pinkish, becoming brownish when cut.

Lamellae crowded, free but reaching the stem, often appearing adnate at first, dull, dirty pinkish brown (approximately vinaceous fawn (*R*)) with a white edge due to tufts of clavate, hyaline hairs, later deep brown, almost black, narrow compared with the thickness of the flesh.

Stipe usually short and thick, 3–4 (–6?) × 2–3·5 cm. thick, whitish, smooth swollen, sometimes abruptly so at the base, which in indoor specimens may be woolly, solid, or rarely with a slight hollow, becoming brownish when cut or rubbed.

Veil thick, forming a soft, swollen median annulus which appears more or less triangular in vertical section, white and sulcate on the upper side, sometimes tinged with fawn colour below.


1
1(a)
2
2(a)
3
3(a)
4
5

Basidia usually two-spored. Spores broadly elliptical, guttulate, rather thick walled, reddish brown by transmitted light, but giving an umber-brown spore print (sepia (*R*)).

Spores 5·7–8·9 × 5·1–7 μ.

REFERENCES

Atkinson, G. F. "The development of *Agaricus campestris*." *Bot. Gaz.* xlii (1906), 241–64.

Brunswik, H. "Untersuchungen über die Geschlecht- und Kernverhältnisse bei der Hymenomycetengattung *Coprinus*." *Bot. Abh.* Heft 5 (1924).

Buller, A. H. R. *Researches in Fungi*, vol. i (1909).

Chevreul, L. Correspondence. *C.R. Acad. Sci.*, Paris, liii (1861), 671–2.

Colson, Barbara. "The cytology of the mushroom, *Psalliota campestris* Quél." *Ann. Bot.* xlix (1935), 1–18.

Duggar, B. M. "Physiological studies with reference to the germination of certain fungus spores." *Bot. Gaz.* xxxi (1901).

—— "On the principles of mushroom growing and mushroom spawn making." *Bull. U.S. Bur. Pl. Ind.* No. 85 (1902).

Falck, R. "Ueber die Sporenkeimung des Champignons." *Mykol. Untersuchungen und Berichte*, Beiheft i (1924).

Ferguson, Margaret C. "A preliminary study of the germination of the spores of *Agaricus campestris* and other Basidiomycetous fungi." *Bull. U.S. Bur. Pl. Ind.* No. 16 (1902).

Hein, I. "Studies on the mycelium of *Psalliota campestris*." *Amer. J. Bot.* xvii (1930), 197–212.

Hoffmann, H. "Untersuchungen über die Keimung der Pilzsporen." *Jb. wiss. Bot.* ii (1860), 197–337.

Lambert, E. B. "The production of normal sporophores in monosporous cultures of *Agaricus campestris*." *Mycologia*, xxi (1929), 333–5.

Murrill, W. A. "Illustrations of fungi. XVIII." *Mycologia*, vi (1914), 161–7.

Peck, C. H. *Icones Farlowiana* (1929).

Sachs, J. *Lehrbuch der Botanik*, i Auflage (1868).

Sass, J. E. "The cytological study of a bispored form of *Psalliota campestris*." *Pap. Mich. Acad. Sci.* ix (1928), 287–98.

—— "The cytological basis of homothallism and heterothallism in the Agaricaceae." *Amer. J. Bot.* xvi (1929), 663–701.

Stewart, F. C. "Is *Psalliota brunnescens* under cultivation?" *Mycologia*, xxi (1929), 41–3.

EXPLANATION OF PLATE III

Fig. 1, 1 *a*. Fuscous cultivated variety.

Fig. 2, 2 *a*. White non-fragrant cultivated variety.

Fig. 3, 3 *a*. Wild species, *Psalliota campestris*.

Fig. 4. Photomicrograph of young gill of white non-fragrant variety showing one- to four-spored basidia. × 140 approx.

Fig. 5. Photomicrograph of young gill of fuscous variety showing two- to four-spored basidia. × 140 approx.

THE PARASITISM OF *MYROTHECIUM RORIDUM* TODE

By N. C. PRESTON, B.Sc.

(With Plates IV and V)

During the summer of 1932 it was noticed that certain pansies in my garden appeared to be slowly dying out. First one shoot and then another gradually withered from the base upwards until by about August the whole plant was almost completely dead.

The stems had rotted at ground level and came away readily from the root which was more or less in a sound condition. On the basal portion of the dead shoots thus detached were numerous small black dots surrounded by a distinct white rim which proved to be the fructifications, sporodochia, of a *Myrothecium* closely approximating to the species usually determined as *M. roridum* Tode.[1]

The constancy with which these fructifications appeared on the dead and dying plants aroused suspicion that the fungus giving rise to them, though generally regarded as a saprophyte, might actually be the cause of the disease. A preliminary test was therefore made, before actually isolating the fungus, by transferring some of the spores from the centre of a sporodochium to the surface of the leaves of a growing potted plant of *Viola cornuta*. Within three days each of the leaves thus inoculated showed definite lesions in the form of dark purple spots, the centre of which rapidly became dry and brownish. In view of these immediately positive results the fungus was isolated in pure culture.

Pure cultures of the fungus were readily obtained by stirring up spores, taken with a sterile needle from the centre of a sporodochium, in a small quantity of sterile water and spreading a drop of this dilution upon the surface of a previously poured agar plate. After forty-eight hours' incubation at 22° C. the isolated growths could be readily picked out and transferred to agar slopes. In even the initial platings there was extremely little contamination though one or two bacterial colonies appeared. These were also isolated and dealt with separately.

The fungus cultures thus isolated were further tested for any bacterial contamination which might possibly have still been overlooked by taking spores from seven-day growths on malt agar and corn-meal agar respectively, suspending these in sterile distilled water and making poured plate cultures from them with plain nutrient agar.

[1] For this identification the author is indebted to Miss E. M. Wakefield.

These plates were kept at 22° C. and examined frequently. Both plates remained free from any visible bacterial colonies for four weeks, by which time there was a very abundant growth of the fungus mycelium upon them.

Bacterial inoculations

As far as could be readily ascertained the few bacterial colonies referred to above appeared to be those of a single organism and, though such colonies were actually very scarce in the initial plates, it seemed just possible that this organism might have been responsible for the lesions produced in the first rough experiment, and a test was therefore arranged as follows. Three shoots of a healthy plant of *Viola* growing in a pot were inoculated with the bacterium, while four others were similarly inoculated with spores from a pure culture of the fungus *Myrothecium roridum*. The inoculum consisted of a suspension in sterile water of spores or bacteria respectively, each being taken from an eight-day-old culture on cherry agar. A drop of the inoculum was placed upon the surface of the plant by means of a sterile platinum loop without any pricking or wounding of the tissues. Each shoot was inoculated upon two isolated internodes. All the shoots thus inoculated with the fungus spores developed characteristic lesions within eight days, while none appeared upon any of those inoculated with the bacterial suspension or elsewhere upon the plant.

Inoculation experiments with the fungus

The second series of inoculations was carried out on September 9, again on a growing *Viola*. Two forms of inoculum were used, one a suspension of spores, the other a suspension of mycelial growth broken up in sterile distilled water. The mycelium for this purpose was taken from the fringe of an actively growing culture. Five stems were inoculated with the spore suspension and three stems with the mycelial suspension, the liquid being applied to the plant surface without any scraping or wounding, the plant being afterwards covered with a bell-jar to maintain a moist atmosphere. At the end of three days all the stems inoculated with the spore suspension showed characteristic purple-brown streaks on the internodes inoculated. Of the three stems inoculated with the mycelial suspension one showed a definite lesion comparable with those on the other five, on the second the lesion was apparent but much slighter, while on the third no lesion was visible. All the remaining uninoculated six stems of this same plant, which could be regarded as controls, remained perfectly normal.

This experiment has been described in detail because, in connection with it, a further test was made to eliminate any possibility of

bacterial contamination being a contributory cause of the lesions produced. This was done by placing a small quantity of each of the suspensions actually used for the inoculation in a sterile Petri dish and adding nutrient agar, previously cooled to 40° C. The plates thus prepared remained quite free from bacterial growth. In view of this result and the fact that the bacteria initially occurring naturally in the sporodochia failed to produce infection, it was considered unnecessary to subject the isolated fungus to any further tests of this nature.

The foregoing experiments were followed up by numerous other similar inoculations both on plants in pots and on detached leafy shoots. The stems, leaves, and basal parts were all subjected to inoculation with positive results. The varieties of *Viola* used were a mauve form, "Maggy Mott", a deep yellow, "Chantryland", and the small blue *V. cornuta*. No marked difference in susceptibility between these varieties was recorded.

It is not proposed to detail all the individual experiments, since the majority were carried out in a manner essentially similar to those already described, but reference to any significant diversions from this method will be made as is necessary.

As regards controls, in the earlier experiments a due proportion of leaves or internodes on the plant used for inoculation were treated with loopsful of sterile water in place of the spore suspension. Later, however, it was deemed satisfactory to regard all uninoculated parts as controls, the inoculated internodes or leaves being carefully marked by loops of thread. Where stem inoculations were made adjacent internodes were not selected for inoculation, the one or more internodes intervening between the inoculated ones thus serving as particularly useful controls. Such control parts invariably remained normal.

Stem and leaf inoculation of growing plants

(*a*) *Spore suspension applied without wounding or pricking of tissue*

Sixteen plants of varieties "Maggy Mott" and "Chantryland" were used in these experiments and ninety-one separate inoculations were made. The results may be summarised as follows:

Internodes inoculated 34; positive infections 25 = 73·5 per cent.
Leaves inoculated 57; positive infections 39 = 68·4 per cent.
Control parts, as described above, all normal.

(*b*) *Tissues pricked before inoculation. Exp.* 14 C

In a single plant, variety Chantryland, the stem and leaf tissues were lightly pricked with a sterile needle and the spore suspension applied in the usual way to the punctured surface.

Five internodes and four leaves (upper surface) were thus inoculated while the same number of each, similarly pricked but treated with drops of sterile water instead of spore suspension, served as controls.

All the inoculated internodes and leaves showed very pronounced lesions within eight days, the discolouring first becoming visible two days after inoculation. The pricked but uninoculated controls showed no discoloration whatever and remained perfectly normal throughout.

It is of interest to note, in connection with this experiment, that the plant described was one of a series of three, the other two of which were inoculated, unsuccessfully, in the usual way, *i.e.* without pricking. The inoculum for the three plants was prepared from an eleven weeks old culture. The effect on the three individual plants is here shown for comparison:

Plant A. 3 internodes inoculated without pricking: result 1 positive, 2 negative.

Plant B. 5 internodes and 4 leaves inoculated without pricking: result all negative.

Plant C. 5 internodes and 4 leaves inoculated after pricking: result all definitely positive.

The fact that all but one of the A and B inoculations proved negative may perhaps be accounted for by the age of the culture, since in a previous experiment, two days before, it proved similarly inactive giving only two positive results out of ten inoculations.

Stem and leaf inoculation of detached shoots

The inoculation of detached shoots of *Viola cornuta* was resorted to at times for convenience.

Only thoroughly healthy strong growing shoots were selected. These were well washed and placed in large covered glass dishes 20 cm. in diameter. The control shoots were sometimes contained in the same dish as those inoculated, sometimes in separate dishes, and in all the experiments here discussed they remained perfectly fresh and normal to the end. Fifteen shoots were used for inoculation.

Internodes inoculated 34; positive $21 = 61{\cdot}8$ per cent.

Leaves inoculated 35; positive $32 = 91{\cdot}4$ per cent.

Shoots kept as controls 11; all parts remained normal.

In one of these experiments the effect of a very dilute spore suspension was compared with that of a heavy suspension with the following result:

Dish *a*. 5 shoots. 15 internodes inoculated with weak suspension: positive 3, after 10 days.
10 leaves inoculated with weak suspension: positive 10, after 10 days.

Dish *b*. 5 shoots. 11 internodes inoculated (heavy suspension): positive 11, after 10 days.
10 leaves inoculated (heavy suspension): positive 10, after 10 days.

Dish *c*. 4 shoots. Uninoculated controls. All parts quite fresh and healthy after 10 days.

In both (*a*) and (*b*) the characteristic discoloration began to be apparent after three days. In (*b*), however, the lesions developed much more rapidly; they were very marked on eight out of the eleven internodes within three days, and all were obviously heavily infected within seven days.

Inoculation of basal parts

Since this fungus first came under observation as the cause of a basal rot it was obviously desirable to see whether a rot of this type could be caused by surface inoculation with the pure culture. Some experiments to determine this were therefore carried out, and these will be best described individually.

Exp. No. 5. Two healthy rooted cuttings A and B of *Viola* var. "Maggie Mott" in pots, were inoculated on February 23, 1933, with material taken from a sixteen days old culture on potato-glycerin agar. A heavy spore suspension was poured down the stem bases of each plant and allowed to run into the surrounding soil and, in addition, some mycelium from the same culture was carefully incorporated with the surface soil in each pot. A third plant C was similarly treated with distilled water to serve as a control, and the three plants were kept together in a cool greenhouse.

On March 27 all three plants, which superficially appeared equally healthy, were lifted and the soil carefully washed from the roots; their appearance was as follows:

Plant A. Main stem with pronounced dark lesion extending from soil level to a height of 2 cm. Over the lower half of this lesion the tissues appeared dark brown, wrinkled and rotting, the upper half of the lesion being a purple-black discoloration. One of the lateral shoots arising 3 cm. above the soil level was similarly blackened over the length of the first internode. The roots and upper aerial parts appeared normal.

Plant B. Apart from slight discoloration the main central shoot appeared sound. Two lateral shoots were characteristically blackened from soil level to a distance of about 2 cm. upwards. Both shoots were rotting at the base. Roots and upper aerial parts were healthy.

Plant C. All parts above and below ground appeared quite healthy. No discoloration apparent.

After examination each plant was transferred to a separate large covered glass dish. When the plants were examined two days later numerous sporodochia of *Myrothecium* were found to have developed upon the basal parts of the stems of plants A and B. Plant C still remained perfectly clean and normal in appearance.

Since the "Maggie Mott" plants were cuttings it seemed possible that they might be more readily susceptible to infection through the callused area than if they had been grown from seed. In further experiments therefore seedlings were used in place of cuttings.

Exp. No. 11. Six healthy *seedlings* of the yellow *Viola* var. "Chantryland", growing in 4-in. pots, were inoculated on August 4, 1934, with a spore suspension of an eight weeks old culture by allowing 3 c.c. of the suspension to run down the lower parts of the stems into the soil. Five similar control plants were treated in the same way with distilled water. The pots were then sunk in soil contained in two large boxes, the inoculated plants occupying one box the controls being in the other, and all were kept in the open.

On August 21 one of the inoculated plants and one control were lifted, washed and examined. Both appeared perfectly healthy and sound at the base. Ten days later (August 31) three more of the inoculated plants were lifted; these also appeared normal, as also did the still growing controls. After examination, these three plants were immediately transferred to covered glass dishes and used for a further experiment described later.

The remaining two inoculated and four control plants were allowed to grow for another seven weeks. When they were examined on October 18 the stem bases and upper parts of the roots of both the inoculated plants showed distinct signs of rotting from the exterior inwards. In addition to extensive discoloration of the stem bases the main tap root, for about 1 cm. from soil level downwards, was brown and discoloured, the discoloration merging gradually into the white firm tissue below.

All the four control plants were quite normal. Their stem bases were healthy, and it was particularly noticeable that the upper part of the root, which in the inoculated plants was badly affected, was here perfectly sound and white.

Exp. No. 11 *a.* The three plants from the previous experiment which had been transferred to glass dishes ten days after inoculation were used. Two of these, in dishes A and B, were further inoculated by running a few drops of fresh spore suspension on to the region between root and stem by means of a pipette, and a small fragment of mycelium was also applied at this point. The plant in the third dish C received no such inoculations and served as a control. On September 10 plant A showed only a scarcely perceptible darkening of the tissues at the point of inoculation, plant B was definitely rotting

at this region, while plant C, the control, remained white and normal.

Exp. No. 12. A single healthy "Chantryland" seedling was inoculated by pouring a spore suspension over the basal part as in previous experiments. A second plant was kept as an uninoculated control. Both plants were washed and examined three weeks after inoculation when their appearances were as follows:

Inoculated plant. Stem base blackening over a distance of 1 cm. from soil level upwards, the discoloration extending into one of the two secondary shoots. The lesion also continued downwards to a point $\frac{1}{2}$ cm. below the soil level where the discoloration gradually diffused into the sound white tissue of the main root. Sporodochia were present in the middle of the main lesion.

Control plant. No lesion or discoloration was present, the normal greenish tinge of the stem base fading into the clean white of the healthy root.

Exp. No. 13. Three healthy "Chantryland" seedlings in 7 in. pots were used. From each of the three plants the soil was washed away at one side by means of a jet of water so as to expose the stem base and adjoining roots. One of the plants, A, was then pricked lightly on the exposed surface with a sterile needle and inoculated with spores from an eleven weeks old transfer by means of a platinum loop. The second plant, B, was similarly inoculated but without pricking, the third, C, was pricked but uninoculated. All three plants were washed and examined eighteen days later.

Plant A showed brown discoloration down the stem and root over 1·5 cm. Tissues below ground were clearly splitting and rotting.

Plant B showed a very slight discoloration at the inoculated region but no signs of rotting.

Plant C. Quite normal.

Recovery of the fungus from artificially inoculated plants

In order to establish the parasitic nature of any fungus conclusively it is necessary to be able to reisolate it from the plant lesions produced by inoculation with the pure culture. This was done successfully with *Myrothecium roridum*. The first plant selected for this purpose was a "Maggie Mott" *Viola* which had been inoculated on August 27, 1932, and in which the characteristic lesions had been produced upon the stem. Eight days after inoculation a small fragment was removed from the drying centre of each of two stem lesions with the point of a flamed scalpel and the fragments of tissue transferred to two cherry agar plates. A pure growth of *Myrothecium roridum*, without any trace of contamination, developed upon each of the plates thus inoculated.

The somewhat rough and ready technique adopted in the foregoing experiment did not, however, absolutely preclude the possibility of the transference of a merely surface growth, or even perhaps of the spores of the fungus, to the agar plates, and a more elaborate procedure was adopted in the following later experiment.

On August 31, 1934, a "Chantryland" seedling was inoculated and kept under a bell-jar until September 4, when definite lesions had appeared on the stems and leaves. One of the stem lesions extended practically the length of an internode, appearing as an elliptical spot, brown and shrunken at the centre and sharply delimited by a purple-black border from the normal green tissue beyond. This internode measured 17×3 mm., the actual dimensions of the lesion being 12×2 mm. The following day a portion of the stem including the infected internode was cut away, immersed for one minute in 0·1 per cent. mercuric chloride, and thoroughly washed in sterile water. Fragments from the infected spot were then removed with a sterile scalpel and plated on cherry agar.

Two infected leaves, one from this same plant and the other from a second plant inoculated at the same time, were also taken. These were similarly treated with 0·1 per cent. mercuric chloride, washed in sterile water, and portions of the diseased tissue transferred to agar plates.

From each of the stem and leaf fragments thus plated a luxuriant mycelial growth was obtained and the typical spore masses of *M. roridum* developed after three days.

Description of the fungus

Beyond the bare descriptions of *Myrothecium roridum* which are to be found in systematic works such as Rabenhorst's *Kryptogamen-Flora*, I have not been able to trace any literature dealing with this fungus, and no previous suggestion of its being actually parasitic seems to have been made. The following brief description may perhaps, therefore, not be out of place.

M. roridum is a member of the Tubercularіaceae, the genus being characterised by the presence of a ring of pure white setae around the margin of the sporodochium. The hyphae, in culture, are hyaline to pale brownish. The spores are straight with rounded ends, continuous and often contain two or sometimes three droplets. They are greenish or of a pale olive tint, appearing jet-black in the mass, and measure approximately $7–8 \times 2\,\mu$.

The fungus grows well at room temperature on many kinds of artificial media, producing a flocculent growth of pure white aerial mycelium. Sporodochia are usually produced in abundance either on the substratum itself or among the loose aerial hyphae. When

lying separate from one another they appear as jet-black dots surrounded by a white rim. Often, however, they coalesce into larger masses and may sometimes form an almost continuous black line around the margin of the culture.

The lesions produced by inoculation of leaves or stems of *Viola* appear first of all as dark purple-black spots or streaks which gradually increase in extent. As the lesion advances the tissues at the centre become dry, shrivelled and brown, the outer margin being sharply delimited from the normal green of the leaf by a deep purple-black band.

Conclusions

From the experiments described it is clear that *Myrothecium roridum* can function as an active parasite. It is evidently able to enter the unwounded tissues of healthy plants of *Viola* and eventually to destroy them. Considering the ease with which infection can be secured and the rapidity with which the lesions develop under suitable conditions, it seems evident that this fungus must be reckoned as one of the possible causes of the dying out of violas under cultivation. This view is supported by the fact that the fungus occurs commonly upon dead or dying *Viola* stems from which it was actually isolated at the beginning of this investigation.

The method of inoculation adopted in the majority of the experiments shows that abrasion or wounding of the plant tissues is not necessary before infection can take place. That such injury would render a plant more liable to infection is, however, indicated by the results obtained in Exps. 13 and 14.

Since the fungus grows readily as a saprophyte not only on dead violas but also on many other kinds of plants, the possibility of infection spreading to violas from various other sources must be taken into account. In this connection it may be mentioned that a culture of *M. roridum* isolated from dead vine stems was found readily to infect healthy living plants of *Viola*.

Summary

Inoculation experiments with the fungus *Myrothecium roridum*, obtained in pure culture from dead *Viola* stems, are described.

A high percentage of infections was secured by applying a spore suspension to the uninjured tissues of healthy *Viola* plants.

The fungus was shown to be able to infect the leaves, stems and hypocotyledonary region of growing plants.

It is concluded that *Myrothecium roridum* must be regarded as a potential parasite under natural conditions.

Footnote. After this paper had been sent to the editors a preliminary account of a serious crown rot of snapdragons caused by *Myrothecium* appeared in *Phytopathology*, xxv (1935), 969—J. J. Taubenhaus: "On a black crown rot of greenhouse snapdragons caused by *Myrothecium roridum* Tode."

Fig. 1

Fig. 2

Fig. 3

Fig. 4

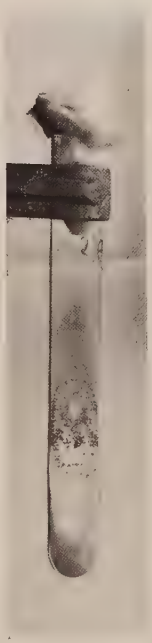


Fig. 5

EXPLANATION OF PLATES IV AND V

Plate IV

Fig. 1. *Viola* seedling, "Chantryland", artificially inoculated with *Myrothecium roridum* showing lesions on stem (*a*) and on leaves (*b*), (*c*). Lesions (*a*) and (*b*) six days, lesion (*c*) ten days after inoculation.

Fig. 2. Part of same plant as fig. 1 enlarged showing sporodochia on the inoculated leaf (*c*).

Plate V

Fig. 3. *Viola* cutting, "Maggie Mott", showing artificially produced lesions (*a*) on the stem, fourteen days after inoculation.

Fig. 4. Extensive lesion on stem of "Maggie Mott" *Viola*, fourteen days after inoculation. Note concentric wrinkling at centre of lesion and the smooth healthy internode above the one inoculated.

Fig. 5. *Myrothecium roridum* Tode. Three weeks' growth on cherry agar.

OBSERVATIONS ON THE RESULTS OF INOCULATING CEREALS WITH THE SPORES OF CEREAL RUSTS WHICH DO NOT USUALLY CAUSE THEIR INFECTION

By THEODORA B. HANES, Ph.D. (Cantab.)

(With 18 Text-figures)

INTRODUCTION

The study of specialisation among the cereal rusts was initiated by Eriksson as early as 1894, and since that time the subject has received much attention. Observations by early workers in different countries on the host range of particular rusts showed unaccountable discrepancies, which became explicable only after the recognition of numerous physiologic forms of the different rusts, the existence of which was first demonstrated in *Puccinia graminis Tritici* by Stakman and Piemeisel in 1917.

Our knowledge of the specialisation and host range of the different cereal rusts is largely based on macroscopic observations of the infected hosts, and consequently the reaction between host and parasite has been defined merely in terms of such visible symptoms as necrosis, flecking, and pustule formation. Such observations give only an incomplete picture of the development of the fungus and its relationship with the host tissues.

The first careful investigations of the intimate relationship existing between host and rust were made by Marshall Ward in 1901 and 1902. Gibson found, in 1904, that chrysanthemum rust could enter various plants on which it does not normally occur. However, no true infections resulted, that is, no haustoria were formed, and by the end of the fourth day the fungus was dead. Killed host cells were observed in the vicinity of these hyphae, and Gibson suggested that the death of the hyphae was due to some poisonous substance emitted by the cells.

Marryat, in 1908, studied two varieties of wheat showing different degrees of resistance to yellow rust, *P. glumarum*. She found that the fungus entered these plants in a normal manner and produced hyphae, but development was restricted owing to the death of host tissue in the infected areas, which cut off the food supply of the parasite. In the variety "American Club", which was the less resistant, the fungus developed further but ultimately death resulted.

Stakman, in 1914 and 1915, studied the development of stem rust

of wheat on immune and susceptible varieties of that host. He was the first to study microscopically the several forms of *P. graminis* on hosts presenting an extreme degree of incompatibility. These studies included oats inoculated with *P. graminis Tritici* and *P. graminis Hordei*; rye, wheat, and barley inoculated with *P. graminis* from *Dactylis glomerata*; and wheat inoculated with *Puccinia graminis Avenae*. The experiments showed that the fungi entered "practically immune" plants in a normal manner, but after the death of a number of host cells they were unable to develop further. Stakman termed such host plants "hypersensitive". He states that immunity and resistance are independent of the state of nutrition of the host,[1] but that the explanation probably lies in the secretion of toxins by host, or parasite, or both.

Newton, in 1922, studying *P. graminis Tritici*, showed that a resistant variety of wheat exhibited the same intolerance to the fungus as described by Stakman (20). She, however, interpreted the early cessation in the development of the fungus as being due to starvation resulting from the death of host tissue.

More recently, Allen, in 1923 and 1926, contributed valuable information regarding the host-parasite relation by means of cytological studies of different physiologic forms of *P. graminis Tritici* on varieties of wheat showing various degrees of susceptibility and immunity. Entry of the fungus was always observed, but even in susceptible varieties the guard cells of the stomata of entry were often killed. The subsequent development of the rust varied. Khapli emmer, a variety of wheat resistant to all forms of *P. graminis*, was inoculated with forms 9, 21 and 27. The first mesophyll cell invaded always collapsed and died but the fungus survived, and occasionally produced minute uredo pustules. In addition to microscopic studies of immune and susceptible varieties of wheat inoculated with *P. graminis Tritici*, Allen, in 1926 and 1927, made cytological studies of Little Club wheat (susceptible) and Malakoff wheat (resistant) inoculated with *P. triticina* P.F. 11.

Ruttle and Fraser, in 1927, studied in detail a resistant and a susceptible variety of oats inoculated with *P. coronata* Corda. They found that the entry of the fungus was similar in both varieties. In Cowra 35, the resistant host, the first cell invaded was killed, but sometimes the fungus continued to develop and eventually produced minute pustules as recorded in *P. triticina* on Malakoff wheat (5), and *P. graminis Tritici* on Khapli emmer (3).

[1] The recent work of Forward, in 1932 (*Phytopathology*, XXII, 493–555), seems to render this view untenable, since she was able to produce changes in infection type with *P. graminis Tritici* P.F. 21 as a result of altering the host metabolism by starvation. For example, after prolonged periods of darkness, hypersensitive areas appeared on hosts which were ordinarily congenial.

The above account shows that we have little information concerning the development of cereal rusts in an extended range of plants. The experiments of Stakman (19, 20) with *P. graminis* apparently provide the only existing account of the development of cereal rusts on hosts on which they are not normally found in nature ("inappropriate hosts"). Most other workers have restricted their experiments to immune and susceptible varieties of the natural host.

The work now presented was designed to supplement existing knowledge concerning this aspect of the problem of specialisation among the cereal rusts.

MATERIALS AND METHODS

Inoculation experiments were carried out with uredospores of the following rusts:

Puccinia triticina Erikss. (brown or leaf rust of wheat).

P. glumarum Tritici Erikss. (yellow or stripe rust of wheat).

P. anomala Rostrup (brown or dwarf rust of barley).

P. coronata Corda (crown rust of oats).

P. graminis Secalis Erikss. (black or stem rust of rye).

In addition, aecidiospores (*P. coronata* Corda) from *Rhamnus catharticus* and *R. Frangula* provided the inoculum for some experiments.

The particular physiologic forms of the fungi used in these investigations are not known, as no work had been done in England on their determination at the time these researches were carried out.

All plants used for inoculation were grown in 4-in. pots in greenhouses and were from one to three weeks old at the time of inoculation. They included wheat, rye, barley, and oats, and, in some experiments, certain grasses in addition.

All the seeds showed excellent germination, except those of *Agropyron repens*, which proved to be unreliable. Young plants of this grass were obtained, however, by potting pieces of rhizome.

In experimenting with hosts which were susceptible to more than one form of rust used, it was necessary to guard against accidental infection. As far as possible, therefore, experiments with different rusts were carried on in different greenhouses. Cultures of the various rusts were established on their appropriate hosts and were maintained in the greenhouses. In a few experiments spores were taken directly from the field and used for inoculation.

For easy reference the details of experiments are set out in tabular form. In these tables the cultural history of the rust used is given in an abbreviated form; for example, G 6 from wheat indicates that the particular rust has been cultured for six successive generations on wheat; G 4 from oats indicates that the rust has been cultured on oats for four successive generations.

At the time of each experiment the germination capacity of the spores used was tested by sowing them on tap water in watch-glasses. Spores frequently showed 100 per cent. germination under these conditions, but it did not necessarily follow from this that even susceptible plants would be heavily infected.

Inoculations were made with a sterile scalpel, only the first leaf of each seedling being inoculated. All inoculated areas were marked with waterproof ink, and the plants were placed in a moist chamber for forty-eight hours after inoculation.

In some experiments the inoculated plants were observed only macroscopically, while in others areas were removed at intervals after inoculation. These were fixed, embedded in paraffin wax (M.P. 50° C.) and sectioned. Cedarwood oil was used instead of xylol for embedding, as the material was then less brittle. Several fixatives were tried, including formol-acetic-alcohol, chrom-acetic, and chrom-acetic-urea of different strengths. Consistently better fixations were obtained with Allen's (2) chrom-acetic-urea, made up in the following proportions: 1 gm. acetic acid, 1 gm. chromic acid, and 0·5 gm. urea in 100 c.c. distilled water.

Sections were cut 10μ in thickness. For staining the following combinations were tried: Heidenhain's iron-alum haematoxylin and orange G; Flemming's triple stain; and diamant fuchsin and light green. The diamant fuchsin and light green combination was best and was used almost exclusively.

INVESTIGATIONS

Section I. ***Puccinia triticina*** Erikss.

In 1907 Pole-Evans published an account of the histology of *P. triticina*. Detailed cytological studies of this rust both on a susceptible and a resistant variety of wheat were made by Allen in 1926 and 1927.

P. triticina was collected at the University Farm, Cambridge, on October 11, 1927. The first transfer was made to seedlings of Wilhelmina wheat in the greenhouse and an excellent crop of spores developed. By successive transfers to Wilhelmina wheat the culture was maintained for two and a half years.

The experiments with *P. triticina* were more numerous than those with other rust forms, since an abundance of spores from greenhouse cultures was always available. Inoculations were carried out on wheat, rye, barley, and oats. The varieties used were Wilhelmina, and occasionally Persian Black wheat; rye of unknown variety; Spratt Archer barley; and Grey Winter oats.

(1) Experiments for macroscopic study of inoculated plants

Inoculations were made on wheat, rye, barley, and oats, but no inoculated areas were fixed. Daily observations were made on all plants. Thirty-five experiments were made, of which twelve representative ones are described in Table I.

Wheat. In the thirty-six experiments in which wheat seedlings were inoculated with spores of *P. triticina* heavy crops of spores resulted in thirty-three; in all but one of these, all the seedlings inoculated were infected. In three experiments no infection resulted, and in two of these the failure of infection was correlated with very low germination of the inoculum.

Rye. In the thirty-four experiments in which rye seedlings were inoculated, macroscopic observation showed that infections resulted in all but five. There was, however, a striking variation in the type of infection from one experiment to another. These experiments fall roughly into the following classes:

(1) Normal infection, as in wheat, resulting in a heavy crop of spores, and without necrosis. Five experiments, in which fifty-six out of fifty-eight inoculated plants were heavily infected.

(2) Weak infection resulting in small pustules with no necrosis. Three experiments in which only four out of twenty-seven plants produced pustules.

(3) Mixed infections in which some leaves produced heavy crops of spores with no necrosis, while others produced few or no spores and showed considerable necrosis. Four experiments in which twenty-nine out of forty plants produced pustules.

(4) Infections resulting in minute pustules on a few leaves accompanied by necrosis, and large necrotic areas on most of the inoculated leaves.

(5) Infections resulting in no pustules but considerable necrosis.

Consideration of the data concerning these experiments has failed to reveal any clue as to the nature of the factors which govern the type of infection on rye.

Barley. In the twelve experiments in which barley seedlings were inoculated, weak infections resulting in minute pustules occurred in three. There was no necrosis on barley.

The total number of barley plants inoculated in these experiments was 111, and of these ten became weakly infected.

In the single experiment (No. 10) in which spores produced on barley were used as the inoculum, no infection resulted on barley seedlings.

Oats. In the eleven experiments in which 111 oat seedlings were inoculated no definite sign of infection was observed, although one plant showed flecking.

Table I. Puccinia triticina. *Experiments for macroscopic study*

Exp. No.	Date	Plants inoculated	Culture used	Results
1	Jan. 9, 1928	Wheat* Rye Barley Oats	G 2 from wheat Germ. 100 %	Wheat 10/10,† good infection Rye 1/10, weak infection Barley 0/10 Oats 0/10
2	Feb. 25, 1928	Wheat Rye	G 3 from wheat Germ. 100 %	Wheat 12/12, good infection Rye 1/12, weak infection. Large necrotic patches on many leaves of rye
3	June 15, 1928	Wheat Rye	G 7 from wheat Germ. 50 %	Wheat 20/20, good infection Rye 0/20 (no necrosis)
4	Aug. 6, 1928	Wheat Rye	G 1 from Wil. taken from P.B. wheat Germ. 78 %	Wheat 16/16, good infection Rye 20/20, good infection. Some rye leaves quite as heavily infected as wheat. No sign of necrosis on rye but a few plants were more definitely flecked than wheat. The flecks were normal in appearance
5	Aug. 28, 1928	Wheat (P.B.) (Wil.) Rye Barley Oats	G 4 from P.B. Germ 75 %	Wheat (P.B.) 10/10, good infection; (Wil.) 10/10, good infection Rye 2/10, poor. Large necrotic patches on some inoculated leaves. Barley 1/10, weak infection. A few minute scattered pustules; first positive infection of barley with *P. triticina* Oats 0/10
6	June 11, 1929	Wheat Rye	G 12 from wheat Germ. 100 %	Wheat 12/12, good infection Rye 12/12, good infection. Slight necrosis. Pustules on both hosts 7 days after inoculation
7	June 26, 1929	Wheat Rye Barley Oats	G 13 from wheat Germ. 100 %	All plants showed definite flecking on 6th day Wheat 20/20, good infection Rye 20/20, good infection, no necrosis on rye Barley, oats, no pustules
8	July 14, 1929	Wheat Rye	G 14 from wheat Germ. 100 %	Wheat 10/10, good infection Rye 0/10, necrosis
9	July 19, 1929	Wheat Rye Oats Barley	G 14 from wheat Germ. 20 %	Wheat 10/10, good infection Rye 5/10, weak infection, necrosis Barley 5/10, weak infection, very small pustules but no necrosis Oats 0/10
10	Aug. 2, 1929	Barley	G 1 from barley (transferred from wheat), very few spores available	No sign of infection after 3 weeks
11	Aug. 7, 1929	Wheat Rye	G 15 from wheat Germ. 100 %	Wheat 10/10, good infection Rye 10/10, good infection. 5 leaves produced large pustules and infection appeared normal: on the other 5, pustules were smaller and there was some necrosis
12	Aug. 25, 1929	Wheat Rye Barley Oats	G 16 from wheat Germ. 100 %	Wheat 9/9, good infection Rye 6/6, good infection; smaller pustules but no necrosis Barley 4/10, very minute pustules but no necrosis Oats 0/10

* Wheat: unless Persian Black (P.B.) is specified, the variety used was Wilhelmina (Wil.).

† The denominator of the fraction represents the number of plants inoculated, the numerator the number which produced pustules. Unless stated there was no sign of flecking on the plants which did not produce pustules.

(2) Experiments for microscopic study of inoculated plants

These experiments were carried out in order to follow microscopically the development of *P. triticina* in wheat, rye, barley, and oats. Seedlings were inoculated as before, and marked areas were removed at intervals and prepared for microscopic examination. The varieties used were the same as before, only one variety of wheat (Wilhelmina) being used for microscopic study.

The details of the experiments are given in Table II.

Table II. Puccinia triticina. *Experiments for microscopic study*

Exp. No.	Date	Plants inoculated	Culture used	Times of fixations: days after inoculation	Remarks on macroscopic appearance of leaf areas at the times of fixation
36	Jan. 9, 1928	Wheat Rye Barley Oats	G 2 from Wil. wheat	2, 3, 5, 7	Flecking on wheat only
				10	Pustules on wheat only
				21	Pustules on wheat only. Necrotic patches on rye which had appeared as small flecks on the 13th day. No sign of infection on barley or oats
37	Feb. 25, 1928	Wheat Rye	G 3 from Wil. wheat	17	Numerous pustules on wheat. Definite necrosis on inoculated leaves of rye, and a few minute pustules on one leaf
38	June 17, 1929	Wheat Rye	G 11 from Wil. wheat	1	Flecking on wheat
				2	—
				3	Flecking on wheat and rye
				4	—
				8	Numerous pustules on wheat. Numerous pustules on some leaves of rye, but fewer and smaller pustules on others accompanied by necrotic patches
39	June 26, 1929	Wheat Rye Barley Oats	G 13 from Wil. wheat	10	Numerous pustules on wheat and rye. No pustules on barley and oats, but definite flecking
40	July 1, 1929	Wheat Barley Oats	G 13 from Wil. wheat	1, 2, 3	No flecking
				4	Flecks on wheat only
				6	—
				10	Numerous pustules on wheat. No sign of infection on barley or oats
41	Sept. 28, 1929	Wheat Rye Barley Oats	G 19 from Wil. wheat	1	No flecking
				2	Faint flecking on wheat only
				4	Definite flecking on wheat only
				6	Wheat as above. A few flecks on rye, but none on barley or oats
				8	No sign of pustules. A few faint flecks on barley
				11	Numerous pustules on wheat. Necrotic patches on rye; no pustules
				17	Numerous pustules on wheat. A few small pustules on rye and large necrotic patches. A few minute pustules on barley and no sign of necrosis. Very faint flecking on oats but no sign of pustules

The data given in the last column of the Table show that the rate of development of *P. triticina* in these plants varied from one experiment to another. The following accounts of the development of the fungus are generalised from the different experiments, but variations in the developmental rate will be indicated.

A study was first made of the progress of the fungus in wheat, the original host. This study provided a basis for comparing its progress in rye, barley and oats.

Development of Puccinia triticina *in wheat*

During the first twenty-four hours many of the spores germinated. Appressoria were formed over the stomata, and the contents of these passed into vesicles in the substomatal cavities. The stomata of entry were not damaged by the fungus. By the second day infecting hyphae were seen growing toward the mesophyll cells, and on the third day many had formed haustoria.

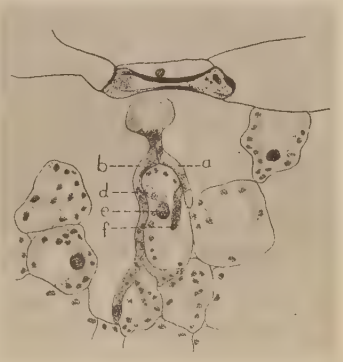


Fig. 1. (×315.)

Fig. 1 (three-day material) shows an entry which is typical of those found in wheat. Here the vesicle is almost empty. The infecting hypha branched into two shortly after its formation. Both branches were closely applied to the walls of mesophyll cells. The first haustorial mother cell (now empty) was cut off at the tip of branch *a*. This produced haustorium *d*, which lay in close contact with the host cell nucleus *e*. Hypha *f* arose just behind the haustorial mother cell. It was full of contents and lay across the invaded host cell. Branch *b* of the infecting hypha was much longer than *a*, but had formed no haustoria *b* at the time of fixation. Hypha *b* was full of contents and showed very clearly the groups of three nuclei which are characteristic of the mycelium of *P. triticina* as noted by Allen (4). There was no disturbance of the surrounding tissues.

After establishing contact with the host tissue by the formation of the first haustorium, the fungus grew rapidly. New hyphae and haustoria continued to develop, and by the fourth day an intimate relation was established with the host. From the seventh day dense wefts of hyphae, in preparation for pustule formation, occurred at intervals below the upper and lower epidermis. Even at this stage the host cells retained their normal appearance. By the eighth day

many pustules had developed. Hyphae in the tissue below the pustules were empty and frequently septate, and large haustoria were present in the host cells. Mesophyll cells in the pustule region were still living, and retained their contents. As noted by Allen (4), a few dead host cells were occasionally found in the vicinity of pustules, apparently crushed by the great mass of hyphae formed.

The development of *P. triticina* on Wilhelmina wheat has been described briefly, since its development on this host agrees closely with that described by Allen for the rust on Little Club wheat, a susceptible variety.

Development of Puccinia triticina *in rye*

Different types of reaction may occur between rye and *P. triticina*. This is clear from the macroscopic observations recorded in Table II. Different types of reaction might occur on different leaves of the same plant, or on different parts of the same leaf.

The variability in the type of infection in rye became evident from the macroscopic observations, some of which are set out in Table I; microscopic study serves to distinguish four different types of reaction in rye, A, B, C and D. These reaction types showed differences in the development attained by the fungus, its relation with the host tissue, and the final fate of the intercellular mycelium.

Type A reaction in rye.

The spores germinated, formed appressoria, and many of the fungi entered the leaves during the first twenty-four hours without damaging the stomata. Vesicles with their infecting hyphae were numerous in the substomatal cavities, and by the second day some of these had formed haustoria in host cells.

Fig. 2 (from two-day material) shows entry into rye at this time. Two empty appressoria can be seen outside a stoma, and two vesicles with their infecting hyphae in the substomatal cavity. Infecting hypha *a* has already formed a haustorium *d*, which lies in contact with a host nucleus, and the terminal haustorial mother cell is now empty. Other infecting hyphae were sometimes branched. No harm was done to the mesophyll cells.

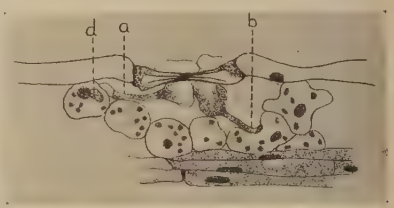


Fig. 2. (×315.)

In one leaf area of rye fixed on the second day, the fungus had already developed several healthy-looking hyphae, a stage of development not usually reached in wheat until the third or fourth day.

In some leaf areas (Exp. No. 38) the fungus was well established by the fourth day, and its relation with the host appeared to be quite congenial.

By the seventh day the intercellular hyphae had spread considerably and formed wefts of mycelium beneath the upper and the lower epidermis. The hyphae were now mostly empty and septate, and large haustoria were seen in the host cells. By the eighth day many pustules had formed. In Exp. No. 38 these were, in general, smaller than those on wheat, but otherwise similar. In Exp. No. 39 large pustules were produced on both surfaces of the leaves.

In type A reaction, the development of *P. triticina* in rye is the same as in wheat, the relation between host and parasite remaining congenial throughout.

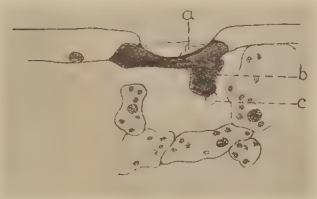


Fig. 3. (×315.)

Fig. 4. (×315.)

Type B reaction in rye.

An examination of leaf areas from several experiments showed that sometimes *P. triticina* made only restricted progress in rye, in contrast to the development which has been described under type A reaction.

The fungus entered as before, but the guard cells of the stomata of entry were usually dead and discoloured. Many vesicles in material fixed after twenty-four hours retained their contents, but these were frequently deeply stained and no infecting hyphae had developed.

Fig. 3 (twenty-four-hour material) shows one of these vesicles. The guard cell, seen in section, is dead, and the shrivelled remains of the appressorium *a* are outside. The vesicle *b* is dead, and very deeply stained. The projection at *c* is probably the rudiment of an infecting hypha.

For three weeks after inoculation, vesicles were seen in the substomatal cavities. Some produced infecting hyphae, and occasionally a haustorium was developed.

A later entry is shown in Fig. 4 (from seventeen-day material).

Here the infecting hypha produced a haustorium *a* in the first host cell encountered. This cell (nucleus, *b*) is now dead and the fungus is considerably shrivelled.

These figures illustrate the maximum development of the fungus in type B reaction. The fungus did not succeed in establishing itself in the host tissue; there was no development after the formation of the infecting hypha, which only occasionally produced a haustorium. It was clear that in this type of reaction an antagonistic relation existed between the fungus and host. Only slight damage was done to the host tissue, since at most only two or three host cells were killed.

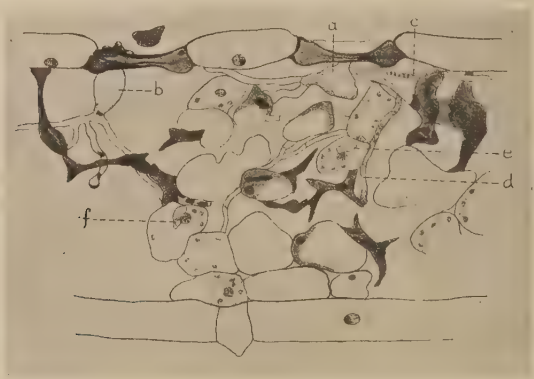


Fig. 5. (×315.)

Type C reaction in rye.

In this type of reaction, as in type B, the relation between rust and host was definitely antagonistic. The fungus, although it did not develop so far as to produce pustules, made considerably more progress than in type B reaction, and consequently larger areas of leaf tissue were damaged, forming visible necrotic patches.

In leaf areas where type C reaction was observed many of the entries showed the type B reaction. Some entries, however, succeeded in establishing an intercellular mycelium, and the vesicles with their infecting hyphae were found empty in the substomatal cavities. If now the mycelium developed, type C reaction was encountered. The characteristics of this type of reaction are shown in Fig. 5 (eleven-day material), where two separate entries are seen at neighbouring stomata. The guard cells are dead. The vesicles *a* and *b* are practically empty. A few intercellular hyphae resulting from these entries can be seen, some (*c* and *d*) retaining their contents while others are

empty. Haustoria can be seen at *e* and *f*, but very few host cells are living. Some are shrunken and so deeply stained that it is impossible to distinguish the contents, while others are empty. Similar empty cells were found by Allen (5), when Malakoff, a resistant variety of wheat, became infected with *P. triticina* P.F. 11. Such infected zones of leaf tissue are local and are separated by zones of healthy cells. The abundance of non-functioning haustorial mother cells is a striking feature of many of the zones exhibiting the type C reaction.

In type C reaction antagonism existed between the fungus and rye, damage done to the host involving considerable areas of leaf tissue. The condition of the fungus itself was weak, and there were no signs of pustules.

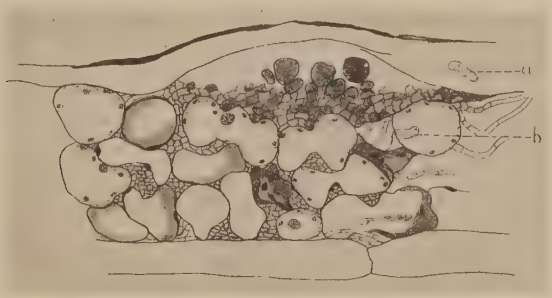


Fig. 6. (×315.)

Type D reaction in rye.

Type D reaction resembles most closely type C, but differs from it in that the fungus succeeded in producing a few minute pustules in the necrotic areas. These appeared in Exp. No. 41, for example, sixteen days after inoculation, whereas pustules appeared on wheat on the eleventh day.

The relation between rye and the rust in type D reaction is illustrated in Fig. 6 (seventeen-day material). Some host cells are empty; others are shrunken and have deeply stained contents; still others are characterised by irregular thickenings of their walls. (Such thickenings were also observed in type C reaction.) They correspond closely to the "swellings" and "warts" described by Allen (5) as occurring outside the infected zones of Malakoff wheat. In rye, however, these swellings were found directly in the infected areas. Fragments of hyphae seen in the tissue have sparse, vacuolated contents, or none at all. Very few haustoria were formed; two are shown, one in an epidermal cell at *a*, and one in a mesophyll cell at *b*. Although small pustules were formed, few spores reached maturity.

Development of P. triticina *in barley*

The rust enters barley as freely as it does wheat and rye. Actual counts were not made, but by the end of the first day vesicles were numerous in the substomatal cavities and the stomata were not damaged. New vesicles continued to develop during the first eight days after inoculation. The fate of most of these became clear from a study of later fixations in which numerous dead and shrivelled vesicles with infecting hyphae were often found in the substomatal cavities, death having occurred before relation was established with the host. These entries were similar to some in type B reaction in rye, except that in barley the guard cells were not killed (cf. Fig. 4).

Barley fixed seventeen days after inoculation in Exp. No. 41 showed zonal infection similar to that described in type D reaction in rye. In barley there were fewer dead cells, indicating that the reaction in barley was less violent than in rye. Only rarely did a few minute pustules develop in barley, and these were considerably aborted. On the rare occasions where *P. triticina* reached the reproductive stage in barley, there was an antagonistic relation between barley and the rust.

Development of Puccinia triticina *in oats*

The rust entered oats freely, forming numerous vesicles in the substomatal chambers. The stomata were not damaged. Frequently two or more vesicles were found in the same cavity, and occasionally infecting hyphae were found.

Some entries seen in oats showed disturbance of the surrounding mesophyll cells although no haustoria had been formed. The rust made little progress in oats, and only once was a small haustorium seen, marking the most advanced stage that the fungus achieved in oats. Leaf areas fixed eight, eleven, and seventeen days after inoculation showed no further development.

Discussion of experiments with *Puccinia triticina*

It seems clear from these experiments that *P. triticina* (in England) is not sharply specialised to wheat. Eriksson (8) found that rye could sometimes be infected with this rust, and Mehta (15), working in England, obtained successful infection on three out of twenty-one seedlings of rye inoculated. These authors were in agreement that rye was subject to "casual infection" by *P. triticina.*

Different types of reaction result when rye is inoculated with *P. triticina.* The phenomena encountered suggest a similarity to the "heterogeneous or X-reaction" described by Stakman and Levine (21) for the types of reaction observed on some wheat varieties inoculated with certain biologic forms of *P. graminis Tritici.*

A further indication that *P. triticina* is not closely specialised to wheat was obtained from Exps. Nos. 5, 9, 12 and 41 (Tables I and II). In these minute pustules were produced on barley.[1] Microscopic examination of inoculated areas removed during the course of Exp. No. 41 revealed infected zones similar to those of type D reaction in rye. Dead mesophyll cells were less numerous in barley, indicating that the reaction was less violent than in rye. The pustules which developed were small and aborted.

Oats was consistently immune to *P. triticina*. Flecking was observed in three out of fifteen inoculations, but no pustules developed. Microscopic examination showed that the rust frequently entered oats, but thereafter made little progress; only one small haustorium was found in the many entries which were examined.

One further point deserves comment. Experiments were carried out to determine whether a sojourn of the rust on rye and barley would increase its virulence on these respective hosts. Spores produced by successful infections on rye with *P. triticina* were used to inoculate seedlings of rye and wheat. The results showed that the virulence was not increased, since these inoculations produced only weak infections on rye accompanied by necrosis, whereas heavy crops of spores were produced on wheat in every trial. Rye was apparently more susceptible to *P. triticina* taken directly from wheat. On the one occasion when pustules on barley produced sufficient spores to be used as an inoculum, these were used to inoculate barley seedlings (Exp. No. 10). No infections resulted.

Section II. ***Puccinia glumarum Tritici*** Erikss.

P. glumarum Tritici is one of five specialised forms of *P. glumarum* described by Eriksson in 1894. Forms of yellow rust occur also on barley, rye, and various grasses. According to Eriksson, the rust on wheat is a specialised form for that host, and does not infect barley or rye. In the United States, however, Hungerford and Owens (12) found that *P. glumarum Tritici* would infect rye "moderately", barley "slightly", and forty-seven wild grasses. They found that oats did not become infected when inoculated with this form.

Mehta (15) showed the strict specialisation of *P. glumarum Tritici* to wheat; barley and rye were not infected by this form.

The histology of *P. glumarum Tritici* has been studied by Pole-Evans (17) and by Marryat (14), and in 1928 Allen published an account of the cytology of *P. glumarum* on *Bromus marginatus* and *Triticum vulgare*.

[1] Prof. H. S. Jackson has informed me that *P. triticina* has been found in the United States, occurring naturally on a wild variety of barley.

Establishment of the cultures

Norka wheat, a variety very susceptible to yellow rust, was used exclusively for the greenhouse cultivation of this rust. Plants of Norka wheat kept on the roof of the Botany School, Cambridge, became infected with yellow rust in October 1927. Successive transfers of the rust were made to Norka wheat in the greenhouse, and for eight months the culture was maintained. In the hot summer of 1928, however, the culture was lost at the end of June. Infected leaves of Little Joss wheat, found at the University Farm on June 30, 1929, provided spores from which another culture was established and maintained in the greenhouse for six months.

Experiments with *Puccinia glumarum Tritici*

Seedlings of Norka wheat, Grey Winter oats, rye (variety unknown), and Spratt Archer barley were inoculated with spores of *P. glumarum Tritici*.

The experiments and results are set out in Table III.

Table III. *Experiments with* Puccinia glumarum Tritici

Exp. No.	Date	Plants inoculated	Culture used	Results
1	May 21, 1928	Wheat Rye Barley Oats	G 4 from Norka	Flecking on wheat on 7th day. Pustules on wheat only on the 10th day. Brownish yellow streaks on barley 14th day, but no indication of infection on oats or rye
2	July 15, 1929	Wheat Rye Barley Oats	G 1 from Norka (new culture)	Flecking on wheat on 7th day. Very small pustules on wheat only on 12th day. No sign of infection on barley, oats or rye
3	Oct. 6, 1929	As in 1 and 2	G 3 from Norka	Moderately good infection on wheat after an incubation period of 3 weeks. No sign of infection on barley, oats, or rye
4	Oct. 30, 1929	As in 1 and 2	G 4 from Norka	As in Exp. No. 3 above
5	Nov. 26, 1929	As in 1 and 2	G 3 from Norka	As in Exp. No. 3 above

In Exps. Nos. 1 and 2, inoculated areas of wheat, rye, barley and oats were removed at intervals after inoculation and prepared for microscopic study. The remaining experiments, Nos. 3, 4 and 5, were carried out for macroscopic observation only.

It seemed clear from the macroscopic observations in the experiments of Table III, that yellow rust of wheat is closely specialised to that host. This was confirmed by the microscopic study of the inoculated plants.

Development of Puccinia glumarum *in wheat*

Within twenty-four hours many spores germinated and formed long germ tubes on the leaves. No entries were found until the second day, although Allen (6) found entries sixteen hours after inoculation; she noted, however, that entry might be delayed for several days. This was also found in my experiments, where new vesicles were found in the substomatal cavities as late as the seventh day. The fungus entered without forming an appressorium, as pointed out also by Pole-Evans (17), and the stomata of entry were not damaged. The germ tubes usually entered at one end of a stoma, but were occasionally found near the centre.

The prolonged initial developmental period of *P. glumarum* under certain conditions is well known. By the fourth day many infections showed no advance beyond the formation of the first haustorium from the infecting hypha; other infections, however, made considerable progress, and thick, non-septate hyphae with numerous nuclei were found among the mesophyll cells.

Leaf areas fixed on the seventh day showed a marked increase in mycelial development. (This coincides with the appearance of flecking on this host.) Hyphae were both branched and unbranched, and they were full of contents. The long, runner-like hyphae described by Allen (6) were frequently seen in leaf areas fixed on the seventh day.

By the tenth day the hyphae were narrower and their contents less dense. Haustoria at this time were mostly of the "hammerhead" type and were found in both epidermal and mesophyll cells. Small pustules had sometimes developed by this time; the epidermis was still unruptured over them, and the hyphae in the pustule regions were considerably drained or else quite empty.

On the fourteenth day numerous pustules had formed on both surfaces of the leaves, and the underlying mesophyll tissue was often almost completely obliterated by the profuse development of hyphae. These were empty and septate. Where the hyphae were less dense, haustoria, large and of various shapes, were present in the host cells. These completely replaced the "hammerhead" type, which were so common on the tenth day. The host cells were impoverished but still living.

Development of Puccinia glumarum Tritici *in rye*

No entries were found in rye until the fourteenth day after inoculation. Earlier material showed many spores of the inoculum on the surface of the leaves; some of these had germinated and formed long germ tubes, which had shrivelled, however, without entering.

In material fixed on the fourteenth day several entries were seen.

The fungus, however, had made little progress and was invariably dead. The guard cells were also dead and discoloured. Fig. 7 (fourteen-day material) shows one of these entries. The empty spore, somewhat shrivelled, lies near the stoma where the fungus entered. As usual with *P. glumarum*, entry was made at one end of the stoma. The guard cell seen in section is dead and discoloured, and closely applied to it is the vesicle *a*, typical of this rust, now empty. In this entry two large infecting hyphae, *b* and *c*, developed. Hypha *b*, soon after its formation, attacked and killed a mesophyll cell bordering on the substomatal cavity. The hypha is now misshapen and deeply stained. The empty haustorial mother cell *d* is seen outside the dead host cell, and it appears that the large haustorium *e* coiled itself about the host nucleus *f*. Both haustorium and nucleus are dead and deeply stained, and in the cell the remains of a few chloroplasts are faintly visible. The hypha is considerably swollen behind the empty haustorial mother cell, a fact which suggests that some food from the host had passed back through the haustorial mother cell before death occurred. After this failure to establish contact with the host, hypha *b* put out a branch *g*, but no infection resulted. This branch *g* is dead and deeply stained like the rest of the fungus. The two host cells in contact with *g* are living and retain their normal appearance. An adjacent cell *h*, though not in contact with the fungus, is dead and deeply stained. The other infecting hypha *c* branched into two just before meeting the host cell *i*, which borders on the substomatal chamber. Both branches of *c* produced haustoria in this cell. It appears that these haustoria, *j* and *k*, arose without the usual formation of haustorial mother cells. Both haustoria are dead, and lie in contact with the nucleus of the dead host cell.

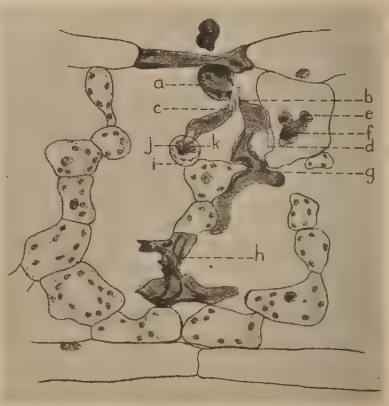


Fig. 7. (×315.)

This entry represents the greatest progress made by *P. glumarum Tritici* in rye; the fungus was short-lived, and only a few host cells were killed.

Microscopic study showed that rye is immune to yellow rust of wheat. For some time after inoculation, although the spores ger-

minated on the leaves, they failed to effect entries. After this delay, however, entries were made, but these were not nearly so numerous as in wheat. Only slight progress was made after entry, the infecting hyphae producing at most one or two haustoria before death. On the rare occasions when this development was attained, a few host cells were killed. The relation between rye and *P. glumarum Tritici* was uncongenial.

Development of Puccinia glumarum Tritici *in barley*

The fungus was first found in leaf areas of barley fixed on the seventh day. As in rye, the guard cells of the stomata of entry were always dead and discoloured. Entries were common in the seven-day material of barley; infecting hyphae had developed but no haustoria had been formed, and in all cases the fungus was dead.

A further stage of development in barley is seen in Fig. 8 (fourteen-day material). The guard cell is dead and the vesicle quite empty. As before, the fungus is now dead, but several host cells were affected before death occurred. Two infecting hyphae developed from the vesicle, *a* and *b* (hypha *b* has been broken in sectioning). Hypha *a* formed a large haustorium *d* in the mesophyll cell *c*, which is devoid of contents. The haustorium has a thick neck; its mother cell *e*, now empty, is closely applied to the wall of the invaded host cell. An adjacent mesophyll cell shows a distinct thickening of its wall. The other infecting hypha *b* extended further but did not produce a haustorium. It penetrated some distance into the leaf tissues, coming into contact with several mesophyll cells. These developed thickened walls where they touched the hypha, but they were still living. Damage done by the fungus was accordingly slight, the area of tissue affected being small.

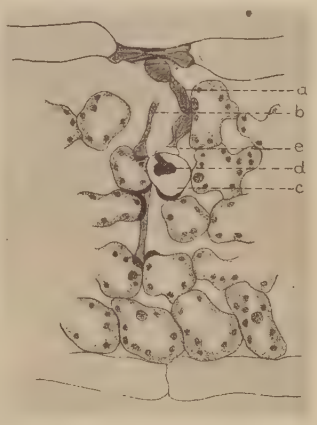


Fig. 8. (×315.)

Other leaf areas of barley taken fourteen days after inoculation showed yellow streaks at this time. It was found that the rust had made considerably more progress in these areas. Thick intercellular hyphae were often found amongst the mesophyll cells, but haustoria were rare.

A section through one of these areas is drawn in Fig. 9 (fourteen-

day material). Hypha *a*, which still retains most of its contents, formed a haustorium at *c*, which lies in contact with the cell nucleus. The empty haustorial mother cell is closely applied to the wall of the invaded cell. Hypha *d* is dead and its contents deeply stained. Some host cells in contact with these hyphae are devoid of contents.

No extensive damage was done by the hyphae in these streaked leaf areas. At most, groups of five or six dead host cells were found. This was the most advanced stage reached by the fungus in barley.

Development of Puccinia glumarum Tritici *in oats*

Entries in oats were less numerous than in rye and barley. As in rye, no entry was seen until several days after inoculation. The guard cells of the stomata of entry were always killed, and the infecting hyphae quickly died.

Fig. 9. (×315.)

Discussion of experiments with *Puccinia glumarum Tritici*

The development of yellow rust in Norka wheat, except for slight points, is in close agreement with the published accounts of Pole-Evans (17), Marrayat (14), and Allen (6). The "intracellular bodies" described by Allen were not found in the infected leaves of Norka. A congenial relation existed between this wheat and the rust; only in regions of heavy spore production were the host cells harmed.

Rye, barley and oats were immune from *P. glumarum Tritici*. Only in one experiment (No. 1) was there any outward sign of infection, some of the inoculated areas of barley becoming streaked.

In these streaked areas in barley the rust had produced thick intercellular hyphae in the mesophyll, but little damage was done to the leaf although some cells were killed and devoid of contents. The reaction between barley and the rust was not violent, and quite

unlike that in the immune wheats such as Einkorn or American Club, studied by Marryat. Usually, the rust made little progress in barley after entry. No relation was established with the mesophyll cells in some infections, while in others slight growth took place after the formation of the first haustorium. The walls of mesophyll cells were always considerably thickened where they came in contact with hyphae.

The rust succeeded also in entering rye and oats; as in barley, the guard cells of the stomata of entry were killed. In rye and oats, however, the mycelia were always short-lived. In rye there was no progress beyond the occasional formation of haustoria, while in oats no haustoria were seen. Damage done to the tissues of these plants was negligible, since only a small number of dead mesophyll cells were found near the points of entry.

Section III.

Puccinia anomala Rostrup = ***Puccinia simplex*** Erikss. & Henn.

Mehta (15) inoculated wheat, rye, barley and *Agropyron repens* with brown rust of barley. He found that the rust attacked only barley. In studying the host specialisation of *Puccinia anomala* Mains (13), in extensive inoculations of grasses, succeeded in infecting only five closely related species of *Hordeum*. Pole-Evans (17) described briefly the histology of this rust on barley.

Establishment of the culture

The culture was established on Spratt Archer barley in the greenhouse from spores collected on the University Farm, Cambridge, on May 11, 1928. The culture was maintained on this variety for nearly two years.

Experiments with *Puccinia anomala*

Spores of this rust were used to inoculate Spratt Archer barley, Grey Winter oats, Wilhelmina wheat, and rye of unknown variety. The experiments are set out in Table IV.

In Exps. Nos. 1 and 4 inoculated leaf areas of all plants were fixed at one, two, three, six and nine days, respectively, after inoculation. In addition, areas of wheat and barley from Exps. Nos. 2 and 5 were fixed fourteen days after inoculation, although these experiments were originally intended for macroscopic observation only. In the other experiments the inoculated plants were kept under observation for one month, no leaf areas being removed.

Table IV. *Experiments with* Puccinia anomala

Exp. No.	Date	Plants inoculated	Culture used	Results
1	July 10, 1928	Barley Wheat Oats Rye	G 3 from barley	Pustules only on barley, after 7 days. No sign of infection on the other plants
2	Sept. 5, 1929	As in 1	G 2 from barley	Faint flecking on barley and wheat after 3 days. Numerous pustules on barley only on 6th day. Wheat definitely flecked after 21 days, but no sign of infection on rye and oats
3	Sept. 9, 1929	Barley Wheat	G 2 from barley	Numerous pustules on barley after 8 days. No sign of infection on wheat at end of 30 days
4	Sept. 17, 1929	Barley Wheat Oats Rye	G 3 from barley	Numerous pustules on barley after 8 days. No infection of the other plants at the end of 30 days
5	Sept. 30, 1929	As in 4	G 4 from barley	Numerous pustules on barley after 10 days. Faint flecks on wheat on 7th day; but no further infection after 30 days. No infection on oats or rye
6	Oct. 15, 1929	As in 4	G 5 from barley	Flecks on barley and wheat on 3rd day. Numerous pustules on barley only, on 8th day. No further infection on wheat, and no sign of infection on rye and oats after 30 days
7	Oct. 28, 1929	As in 4	G 6 from barley	Flecks on barley and wheat on 8th day. Pustules on barley only, on 11th day. No further infection on wheat, and no signs of infection on rye and oats after 30 days
8	Nov. 16, 1929	As in 4	G 7 from barley	On 11th day—numerous pustules on barley without previous flecking, but wheat flecked at this time. No further infection on wheat, and no signs of infection on oats or rye after 30 days
9	Nov. 23, 1929	Barley Wheat	G 7 from barley	Fair infection on barley, pustules appearing on 17th day. No sign of infection on wheat after 30 days

Development of Puccinia anomala *in barley*

By the end of the first day most of the spores had germinated and several entries were found. Germ tubes were occasionally seen which had branched into two on the leaf surfaces. Appressoria were formed over the stomata, and the contents of these passed into narrow elongated vesicles in the substomatal cavities, lying parallel to the guard cells. Infecting hyphae were not seen until the second day; these developed from one or both ends of the long vesicles.

By the end of the third day the fungus had made considerable progress. Delicate intercellular hyphae ramified in the host tissue, and numerous haustoria were present in the mesophyll cells. The infected zones were more or less local, as they were in material fixed six days after inoculation. Occasionally long, runner-like hyphae passed from one infected zone to another, often encountering as many as twelve cells before forming a haustorium. Some pieces of barley

leaf fixed on the sixth day showed various stages in reproductive activity. Wefts of mycelium in preparation for pustule formation were found under the epidermis, and pustules were sometimes present on both surfaces of the leaves. Hyphae in the pustule regions were empty and septate, and numerous uninucleate haustoria, more or less drained, lay in the host cells. The host cells retained their living contents. Throughout the development of the rust the relation was congenial.

Development of Puccinia anomala *in wheat*

The leaf areas of wheat fixed at intervals in Exps. Nos. 1 and 4 showed no entry of the fungus, in spite of the fact that many of these areas were flecked at the time of fixation. It seems clear that flecking here was not due to the fungus. Many spores had germinated on the leaves, but the germ tubes shrivelled without forming appressoria.

In Exps. Nos. 2 and 5, however, in which flecking of wheat was also observed, areas removed fourteen days after inoculation showed entry of the fungus. Where entry had occurred the guard cells were always dead. Sometimes no infecting hyphae were developed from the vesicles, but frequently one or two were found. The vesicles and infecting hyphae were always quite empty and much shrivelled. Only once was a small haustorium seen.

However, rather large groups of cells were sometimes killed around points of entry. Mycelium could not be seen in these dead areas, nor could haustoria be distinguished, as the cells were shrunken and deeply stained. The empty vesicles found in the substomatal chambers alone suggested that the damage to the leaf tissue was caused by the fungus.

Development of Puccinia anomala *in oats*

Germinating spores were seen on the surface of the leaves at all times of fixation, that is up to the ninth day after inoculation, but only occasionally was entry achieved. When this occurred the guard cells were killed. No infecting hyphae developed and the mesophyll cells around the vesicles appeared normal.

Development of Puccinia anomala *in rye*

A few entries were observed on the second day after inoculation. These were similar to entries found in oats at this time. The guard cells of the stomata of entry were always killed. No infecting hyphae developed from the vesicles, and the surrounding mesophyll tissue showed no signs of disturbance. No further progress was made in rye.

Discussion of experiments with *Puccinia anomala*

The experiments indicate a close specialisation of the rust to barley, a congenial relation being maintained between barley and the rust throughout its development.

P. anomala occasionally entered wheat, oats and rye, and when this occurred the guard cells of the stomata of entry were always killed. Only wheat, among these plants, showed flecking, but this also occurred on some leaves in which entries had not been made. A single small haustorium was found in wheat, and here the invaded host cell was not killed. Mesophyll cells in contact with infecting hyphae had thickened walls. In some entries in wheat it was clear that a violent reaction had taken place resulting in the death of groups of mesophyll cells, in addition to the guard cells. Hyphae could not be seen amongst these dead tissues surrounding such entries. The damage done to wheat was never extensive.

Entries were fewer in oats and rye than in wheat. No infecting hyphae developed, and no damage, apart from the death of guard cells, resulted. Not a single dead mesophyll cell was seen in oats or rye.

Section IV. ***Puccinia coronata*** Corda

In 1922 Hoerner carried out extensive inoculation experiments on barley, rye, wheat, and many grasses, using crown rust of oats from different localities. The results showed that crown rust of oats in the United States has an extensive host range under greenhouse conditions, since, in addition to successful infections of many varieties of oats, a number of grasses and three varieties of barley became infected. Flecking was observed on *Lolium italicum* (but not on *L. perenne*), and also on rye and wheat, but no pustules were formed on these plants.

A brief account of the histology of this rust was given by Pole-Evans (17). Ruttle and Fraser (18) published a detailed account of the cytology of *Puccinia coronata* on Banner oats, a susceptible variety, and on Cowra 35, a variety which showed different degrees of resistance.

Establishment of the uredospore culture

Grey Winter oats were used as the host for the greenhouse cultivation of the rust. The original source of the rust was from infected oats found at the University Farm, Cambridge, on November 14, 1927. The culture was maintained in the greenhouse for two years.

PART A. INOCULATION WITH UREDOSPORES OF *PUCCINIA CORONATA*

On May 29, 1928, seedlings of the following plants were inoculated with uredospores: Grey Winter oats, Wilhelmina wheat, Spratt Archer barley, rye, *Lolium perenne* and *L. italicum*.

Marked leaf areas from all these plants were removed one, two, three, five, seven, ten and fourteen days, respectively, after inoculation, and prepared for microscopic study.

Macroscopic observations of the inoculated plants

Flecks appeared only on oats and wheat on the fourth day. By the sixth day barley showed signs of flecking, but these were faint, compared with those on oats and wheat, those on wheat being most distinct. By the tenth day oats, wheat and barley were definitely flecked, but there were no signs of flecking on the other plants. On the fourteenth day, when the final fixations were made, pustules were numerous on oats.

Inoculated leaf areas, which had not been removed during the course of the experiment, were kept under observation for one month. No pustules developed on wheat or barley, and there was no sign of infection on rye, *Lolium perenne*, or *L. italicum*.

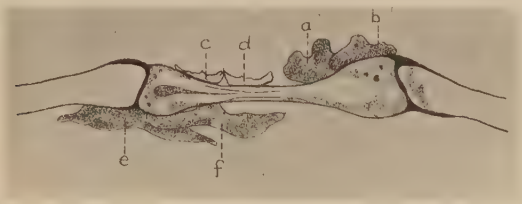


Fig. 10. (×630.)

Development of Puccinia coronata *in oats (from uredospores)*

The spores germinated by the end of the first day and formed appressoria over the stomata. The appressoria were either regular in outline or lobed as described by Ruttle and Fraser (18). Stomata of entry were not damaged. The appressorial contents had mostly passed into vesicles in the substomatal chambers, and by the end of the first day these had already assumed the elongate form characteristic of this rust. It was not unusual to find two or more vesicles in one substomatal cavity, as, for example, in Fig. 10 (*e*, *f*) (twenty-four-hour material), which shows four appressoria.

The rust made little progress during the next four days, though sometimes long, delicate infecting hyphae developed from one or both arms of the vesicles, leaving the latter empty. A median septum could often be seen in the vesicle. The infecting hyphae sometimes penetrated for some distance into the host tissue before forming a haustorium, though often the first host cell encountered was invaded.

At the end of the first week no extensive mycelium had developed, only a few delicate hyphae being observed among the mesophyll cells. By the tenth day, however, the stages preparatory to pustule formation were observed. Hyphae, dense with contents, were now massed

below the upper and lower epidermis, the hyphae in the central parts of the leaves being almost empty. Haustoria in these regions were drained; they were seen in both mesophyll and epidermal cells. By the fourteenth day numerous sporing pustules had formed on both surfaces of the leaves.

Throughout the development of the rust its relation with the host was completely congenial. The rate of development of *P. coronata* in Grey Winter oats in this experiment was considerably slower than its development in Banner oats described by Ruttle and Fraser (18).

Development of Puccinia coronata *in wheat, rye, barley,* Lolium perenne *and* Lolium italicum

The stage of development reached by the rust in all these plants was approximately the same.

By the end of the first day many spores had germinated, and formed numerous appressoria over the stomata; the appressoria were usually

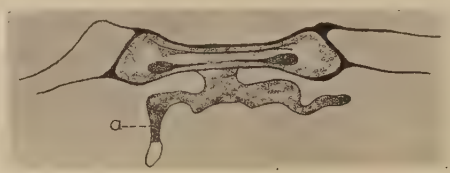


Fig. 11. (×630.)

empty, having formed large vesicles in the substomatal cavities. The vesicles were of the typical elongate form as found in oats, and infecting hyphae (*a*) had already begun to develop in many entries. This condition is shown in Fig. 11 (for rye).

Similar entries were also common in barley, *Lolium perenne* and *L. italicum* fixed at the same time. The stomata entered by the fungus were not damaged at this stage.

After entry, the fungus made little progress. Later fixations showed that the vesicles with their short infecting hyphae had shrivelled in the substomatal cavities before reaching the mesophyll. This was common in all leaf areas fixed after the fifth day, and the guard cells of the stomata of entry had been killed. Only occasionally in these plants did infecting hyphae reach the mesophyll, when a few cells were either killed or their walls were thickened if in contact with the hyphae.

This is shown in Fig. 12, drawn from wheat fixed on the seventh day. Three germ tubes reached the stoma, but only one of these, *a*, entered, leaving outside the remnants of the appressorium. The other

two appressoria, *b* and *c*, appear to be drying up. The vesicle which developed from *a* produced two infecting hyphae, *d* and *e*, but no haustoria were formed. Hypha *e* put out several branches which passed between the mesophyll cells. No cells were killed, but their walls were often thickened where in contact with the hyphae. The fungus itself was somewhat shrivelled.

Only a single haustorium was observed in these plants. This was found in a leaf area of barley fixed on the seventh day. This entry was the furthest development of *Puccinia coronata* in barley, and in forming one small haustorium, it exceeded the progress of the rust in wheat, rye, *Lolium perenne* and *L. italicum*. The damage done to the leaf tissue was always negligible.

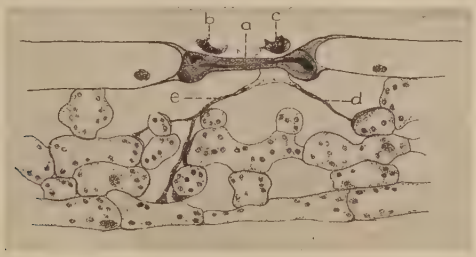


Fig. 12. (×315.)

Discussion of results with uredospores of *Puccinia coronata* Corda

The microscopic study of this rust in oats showed that in its normal development there was a congenial relation between host and parasite.

The rust gained entry into wheat, barley, rye, *Lolium perenne* and *L. italicum*, without at first damaging the stomata. Its subsequent progress was extremely limited, and only once did an infecting hypha produce a haustorium. This occurred in barley. Sometimes a few dead mesophyll cells were seen in contact with the infecting hyphae. Damage done to these plants was negligible and they were immune to attack from *Puccinia coronata* Corda.

PART B. INOCULATIONS WITH AECIDIOSPORES FROM *RHAMNUS CATHARTICUS* AND *RHAMNUS FRANGULA*

In the experiments with aecidiospores from *R. catharticus* and *R. Frangula* the method of inoculation was the same as in the previous experiments.

Infected bushes of both species of *Rhamnus* were found near Cambridge. Fresh spores were used to inoculate Grey Winter oats, Wilhelmina wheat, Spratt Archer barley, rye (unknown variety), *Lolium perenne* and *L. italicum*.

For each experiment a fresh supply of aecidiospores was obtained from the field. The spores invariably showed 100 per cent. germination in tap water when used for inoculation.

The experiments are summarised in Table V.

Table V. *Experiments with aecidiospores from* Rhamnus

Exp. No.	Date	Plants inoculated	Source of aecidiospores	Results
1	June 11, 1929	Oats Wheat Barley Rye *L. perenne* *L. italicum*	*R. catharticus*	Faint flecks on wheat and rye 6 days after inoculation. After 30 days no sign of infection of the other plants and no further development in wheat and rye; no sign of pustules
2	June 18, 1929	As above	*R. catharticus*	As in Exp. No. 1
3	July 4, 1929	As above	*R. Frangula*	No flecking on any of the plants. No sign of infection at the end of 30 days
4	July 17, 1929	As above	*R. catharticus*	Faint flecks on wheat on the 6th day and on oats on the 7th. One month after inoculation some leaves of oats and wheat still showed flecking but no pustules. No sign of infection on the other plants

In Exps. Nos. 1 and 2 marked leaf areas were removed at intervals from all the inoculated plants and prepared for microscopic study. The times of fixation were as follows: in the former one, three, five, seven and fifteen days after inoculation; in the latter two, three, seven, ten and fifteen days after inoculation.

The results of these experiments were unexpected, in that no pustules were produced on oats by aecidiospores from *Rhamnus catharticus*. Further consideration of these observations will be postponed until the discussion at the end of this section.

Microscopic observations on plants inoculated with aecidiospores from *Rhamnus catharticus*

Examination of the inoculated leaf areas showed that entries resulting from germinating aecidiospores are essentially the same as those resulting from the uredospores of *Puccinia coronata* Corda, described above. So far as is known, this study constitutes the first detailed demonstration of the method by which aecidiospores effect entry into the plant.

Although many spores germinated during the first twenty-four hours, germinating spores were observed on leaves as late as two

weeks after inoculation. The fungi entered the leaves of all plants inoculated. Appressoria were formed over the stomata; these were always smooth in outline, and never lobed as were often the appressoria which developed from uredospores of *P. coronata*. The vesicles, however, were almost identical with those which developed from the uredospores. Although the progress made after entry was slight, it varied in the different plants which were inoculated.

Development of the fungus in oats

Many aecidiospores germinated during the first twenty-four hours and formed appressoria over the stomata. Vesicles were found in the substomatal cavities, but no infecting hyphae had developed. The stomata of entry were not damaged.

Fig. 13. (×630.)

Fig. 14. (×630.)

The method of entry is shown in Figs. 13 and 14, drawn from twenty-four-hour material. In Fig. 13 a vesicle (*c*) is just being formed, the large appressorium (*b*) over the stoma still retaining most of its contents. Part of the shrivelled germ tube (*a*) is still attached to the appressorium.

The vesicle shown in Fig. 14 is fully developed and has the characteristic shape of the vesicle of crown rust of oats. Rudimentary infecting hyphae (*a*, *b*) can be seen, one at each arm of the vesicle.

In five-day material the fungus was still at the same stage, although the vesicles generally showed signs of shrivelling. Occasionally infecting hyphae had developed and had reached mesophyll cells, but the vesicles were always empty and shrunken and the infecting hyphae considerably shrivelled. No haustoria had developed. One

of these entries is shown in Fig. 15. Here the guard cells are still living but are somewhat discoloured. The empty germ tube (*a*) and appressorium (*b*) are seen outside and the shrunken vesicle (*c*) in the substomatal chamber. Only one infecting hypha (*d*) has developed. This reached a mesophyll cell (*e*), which is dead and deeply stained. It is impossible to distinguish the contents, and therefore it is not known whether a haustorium was formed.

Similar entries were found in leaf areas fixed on the tenth and fifteenth day after inoculation, but no progress beyond the stage shown in Fig. 15 had been made. In all the leaf areas of oats not a single haustorium was seen and there was no trace of an intercellular mycelium.

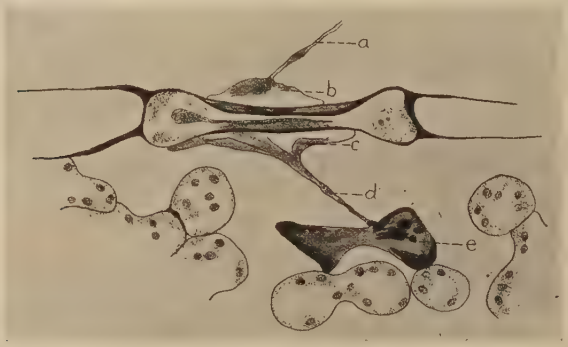


Fig. 15. (×630.)

Development of the fungus in Lolium perenne *and* Lolium italicum

The spores germinated, forming appressoria and vesicles. These were numerous in both species of *Lolium* by the end of the first day and were similar to those described for oats. No further development occurred after entry. Leaf areas fixed on the fifteenth day showed empty, shrivelled vesicles but no infecting hyphae, although rudiments of these were sometimes apparent.

Development of the fungus in wheat

Vesicles were quite numerous in wheat by the end of the first day. Frequently two or more were found in the same cavity, and no damage to the stomata resulted. No infecting hyphae developed beyond the rudimentary stage, as in *Lolium perenne* and *L. italicum*.

Inoculated areas of wheat which showed flecking at the time of fixation revealed no development of the fungus after entry. The

formation of vesicles marked the maximum development in wheat. Vesicles were found on the fifteenth day after inoculation, but at this time they were always shrivelled.

Development of the fungus in barley

Leaf areas of barley showed numerous entries; frequently as many as four vesicles were found in one substomatal cavity.

An unusual entry found in barley is drawn in Fig. 16 (five-day material). The remains of two appressoria (*a*, *b*) are seen over the stoma. Separate entries were made, but these appear to have fused

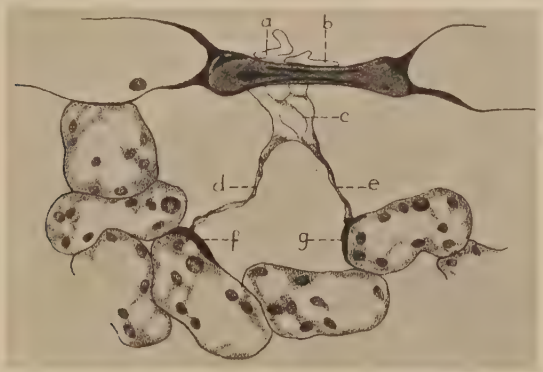


Fig. 16. (×630.)

to form a large single vesicle (*c*) in the cavity. This is now empty and somewhat shrivelled. Two infecting hyphae (*d*, *e*) were produced, both of which reached the mesophyll but did not form haustoria. The walls of the mesophyll cells are thickened (*f*, *g*) where they are in contact with the hyphae, but both cells are living.

The furthest development of the fungus in barley was seen in a five-day fixation in which an infecting hypha formed a large haustorium in a mesophyll cell. No further development took place. This was the only haustorium seen in barley. There was no trace of an intercellular mycelium in barley.

Vesicles were found as late as the fifteenth day, the guard cells of the stomata of entry being usually dead. The vesicles at this time were either empty or completely shrivelled.

Development of the fungus in rye

Entries were common in rye by the end of the first day; these were similar to those described in oats. As late as the fifteenth day, however, germinating spores were still seen on the leaves.

Rye only, of all the plants inoculated with aecidiospores, showed traces of an intercellular mycelium. This was found in several areas fixed on the fifteenth day. The mycelium was scanty and consisted of only a few meagre hyphae. There were no signs of the entries from which the mycelia had developed, except that collapsed stomata were found in the infected regions. Large haustoria were seen in some cells, the latter being usually empty. These haustoria were of distinctive

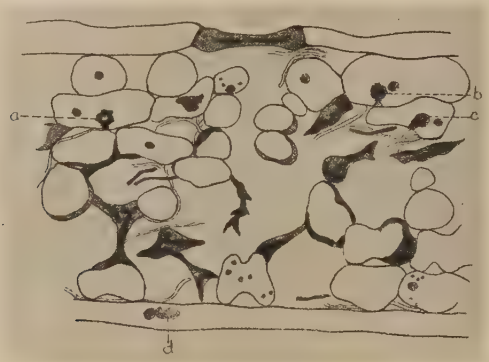


Fig. 17. (×315.)

type with thick necks and enclosed in striated sheaths of irregular margin. Some haustoria were transparent, with deeply stained sheaths, while others were deeply stained and the sheaths were clear, colourless and plainly striated. Haustoria similar to these found in rye have been described by Allen (2), occurring in infections of *Puccinia graminis Tritici* on Mindum wheat, a resistant variety.

Fig. 17 (fifteen-day material) is drawn from a section through one of these infected regions in rye in which an intercellular mycelium had developed. Most of the cells are dead and empty; in some the cell walls are very much thickened, while other cells have collapsed and are deeply stained. The hyphae and haustoria are dead. Haustoria are present in the mesophyll cells at *a*, *b* and *c*. These are stained in different ways and show the characteristic thick necks. Only haustorium *d*, which is in contact with the host nucleus, appears to

be living. In such infections there was an antagonistic reaction between rye and the fungus.

Discussion of inoculations with aecidiospores from *Rhamnus* spp.

The three experiments outlined in Table V show that the aecidiospores from *R. catharticus* failed to produce pustules on any of the plants inoculated, although flecking occurred on wheat and rye in two of the experiments (Nos. 1 and 2), and on wheat, rye and oats in the other experiment (No. 4). In the experiment (No. 3) with aecidiospores from *R. Frangula* there were no signs of infection on any of the inoculated plants.

The microscopic study, which was restricted to plants inoculated with aecidiospores from *R. catharticus*, has shown that the failure of the fungus to produce successful infection was not due to failure to make entry, since entries were numerous in all these plants.

Although the appressoria formed by the germinating aecidiospores were always smooth in outline, and never lobed as were some appressoria from germinating uredospores of crown rust of oats, the vesicles which developed had the characteristic form peculiar to *Puccinia coronata* Corda.

The microscopic study showed, however, that no true infection of oats resulted from inoculation with aecidiospores from *Rhamnus catharticus*. Entries were made and a few infecting hyphae developed, but no haustoria were formed.

In wheat, *Lolium perenne* and *L. italicum* the progress of the fungus was similarly restricted, no more development being made than in oats. It seems, therefore, that the flecks seen on wheat and oats were not due to the fungus.

Although barley showed no flecking, the fungus made slightly more progress in this plant than in oats and wheat. The development of infecting hyphae was rather more frequent, and in one entry a haustorium was formed in a mesophyll cell. There was, however, no further development.

Only in rye did the fungus develop so far as to produce an intercellular mycelium. The mycelium was scanty and it was always clear that there was an antagonistic relation between rye and the fungus, since the hyphae and most of the haustoria were dead, as well as many of the mesophyll cells in the infected zones.

All the plants inoculated were immune to aecidiospores obtained from *Rhamnus catharticus*. It is evident, therefore, that the rust was not the aecidial stage of the form of *Puccinia coronata* Corda, which was cultivated on Grey Winter oats in the greenhouse. It doubtless belonged to one of the many other physiologic forms of *P. coronata*.

Section V. ***Puccinia graminis Secalis*** Erikss.

P. graminis Secalis is one of six specialised forms of black rust recognised by Eriksson (7). According to him, this rust attacks rye, barley, *Agropyron repens* (couch-grass), and other grasses in Sweden, but not wheat or oats.

Stakman and Piemeisel (23) found that *Puccinia graminis Secalis* from *Agropyron repens* infected rye, barley, *A. repens*, and some other grasses, but oats and wheat were rarely infected. Mehta (15) was able to infect rye, barley, *A. repens*, and one variety of wheat (Red Sudan), but not oats.

Microscopic studies of *Puccinia graminis* have been confined almost exclusively to *P. graminis Tritici*, the black rust of wheat. A short account of the histology of *P. graminis Tritici* was published by Pole-Evans (17). Stakman (19) studied the histology of this rust on a susceptible variety of wheat (Minnesota No. 163) and on a resistant variety (Khapli). Further, he (20) made microscopic studies of oats inoculated with *P. graminis Tritici*, wheat inoculated with *P. graminis Avenae*, oats inoculated with *P. graminis Hordei*, and rye, wheat and barley inoculated with *P. graminis* from *Dactylis glomerata*.

There remain to be mentioned in connection with *Puccinia graminis Tritici* the study by Newton (16) of the development of the rust in a susceptible and a resistant variety of wheat, and the cytological studies by Allen (1, 2, 3).

Establishment of the culture on *Agropyron repens*

Uredospores were collected at Cambridge and greenhouse cultures were established on rye and couch-grass.

For some experiments uredospores for inoculation were taken directly from couch-grass out of doors, but for the later experiments they were obtained from the greenhouse cultures.

Experiments with *Puccinia graminis Secalis*

The plants inoculated with this form of black rust were as follows: *Agropyron repens*, rye (unknown variety), Spratt Archer barley, Wilhelmina and Norka wheats, and Grey Winter oats.

The experiments with *Puccinia graminis Secalis* are given in Table VI.

In Exp. 4 marked leaf areas were removed at intervals and prepared for microscopic study; the fixations were made one, three, five, seven and ten days after inoculation. In the remaining six experiments the plants were kept under observation for one month.

The data in the last column of the table may be summarised as follows:

Couch-grass. In five out of the seven experiments medium to heavy infections resulted; in one experiment there was a weak infection, and in one experiment no infection. Out of a total of sixty-nine plants inoculated (omitting Exp. No. 4) thirty-nine produced pustules.

Table VI. *Experiments with* Puccinia graminis Secalis

Exp. No.	Date	Plants inoculated	Culture used	Results
1	June 6, 1928	Couch-grass Rye Barley Wil. wheat Oats	From couch-grass in open	Couch-grass 9/12,* fair infection Rye 8/10, fair infection No sign of infection on: Barley 0/10 Wheat 0/10 Oats 0/10
2	Oct. 3, 1928	As in 1	From couch-grass in open	Couch-grass 0/17, flecking Rye 6/12, weak infection Barley 0/15, flecking Wheat 0/14 Oats 0/12
3	July 23, 1929	As in 1	G 1 from rye (greenhouse culture)	Couch-grass 10/10, very good infection Rye 10/10, very good infection Barley 0/10 Wheat 0/10 Rye 0/10
4	Aug. 25, 1929	Couch-grass Rye Barley Wil. wheat Norka wheat Oats	G 2 from couch-grass (greenhouse culture)	Areas removed from plants and fixed. There were numerous pustules on rye on 7th day and on couch-grass on 10th. Weak infection on barley after 21 days. No sign of infection on Wil. wheat, Norka wheat, or oats
5	Aug. 25, 1929	As in 4	G 3 from rye (greenhouse culture)	Couch-grass 10/10, excellent infection Rye 10/10, excellent infection Barley 2/10, very weak (after 21 days) Wil. wheat 1/10, very weak Norka wheat 0/10 Oats 0/10
6	Sept. 6, 1929	Couch-grass Rye Barley Wil. wheat Oats	G 4 from rye (greenhouse culture)	Couch-grass 8/10, good infection Rye 11/11, excellent infection Barley 0/10 Wil. wheat 0/9 Oats 0/7
7	Sept. 29, 1929	Couch-grass Rye Barley Wil. wheat Oats	G 5 from rye (greenhouse culture)	Couch-grass 2/10, weak infection Rye 3/10, weak infection Barley 0/10 Wil. wheat 0/10 Oats 0/10

* The denominator of the fraction represents the number of plants inoculated, the numerator the number which produced pustules.

Rye. Medium to heavy infections resulted in five out of the seven experiments, and weak infections in the other two. Out of a total of sixty-three plants inoculated (omitting Exp. No. 4) forty-eight produced pustules.

Barley. Very weak infections on barley were observed in two experiments (Nos. 4 and 5), and flecking in one experiment (No. 2). There was no sign of infection in the other experiments. Out of a total of sixty-five plants inoculated (omitting Exp. No. 4) only two produced pustules.

Wilhelmina wheat. Out of a total of seventy-three plants inoculated in the seven experiments, only a single plant (in Exp. No. 5) produced pustules, and these were few and minute. *Norka wheat* and *oats* were consistently immune.

These results are in agreement with the observations of Mehta (15), who found that couch-grass and rye were favourable hosts for *P. graminis Secalis*, and that Spratt Archer barley showed only weak infections. Mehta, and other workers, however, obtained good infections on other varieties of barley with *P. graminis Secalis.* Mehta also obtained weak infections on one variety of wheat, as was observed in my experiments with one plant of Wilhelmina wheat. Various workers have found that oats is consistently immune to this form of black rust.

Microscopic observations of plants inoculated with *Puccinia graminis Secalis*

Development of Puccinia graminis Secalis *in* Agropyron repens

Under the conditions of the experiment the rust made little progress in couch-grass during the first five days. By the end of the first day numerous appressoria had been formed over the stomata but only about 1 per cent. had formed vesicles and no infecting hyphae were seen at this time. Even on the third and fifth days comparatively few entries were found although appressoria were still numerous over the stomata. Usually, whether these stomata were penetrated or not, the guard cells were dead, and had lost their affinity for the diamant fuchsin stain. Stomata similar to these were also found by Allen (5, 3) in infections of wheat with *P. graminis Tritici.*

Sometimes guard cells were seen in which part of the protoplast was normally stained and part seemed dead and shrunken. This condition was seen only sometimes where the fungus had entered, as shown for example in Fig. 18 (seven-day material). The shrivelled germ tube (*a*) and appressorium (*b*) are seen outside. The fungus entered at one end of the stoma, forming a large vesicle (*c*). A single infecting hypha (*d*) was produced which ran more or less parallel to the leaf surface, and gave rise to a large haustorium (*e*) in the epidermal cell adjacent to the stoma of entry. The part of the guard cell nearest the haustorium is dead and shrunken (*f*), whereas the end where the fungus entered is apparently undamaged. The formation

of the first haustorium in an epidermal cell as in this entry was found to be general in infections with *P. graminis Secalis*.

From the seventh to the tenth day all stages of reproductive activity could be seen. When the final fixations were made (ten days after inoculation) numerous pustules had been formed on both surfaces of the leaves. Most of the intercellular hyphae in the pustule regions were quite empty, and were frequently septate. They were so densely packed that the outlines of some of the cells were completely obscured. Haustoria were rare in host cells immediately below pustules, and when present they were drained.

Apart from the death of guard cells, the relation between couch-grass and the rust throughout its development was congenial.

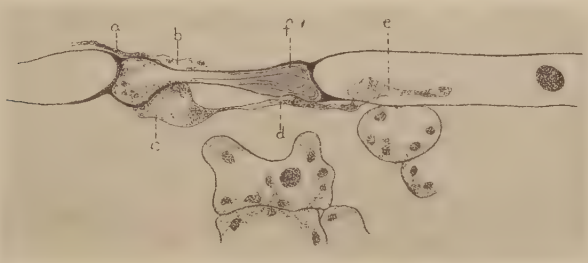


Fig. 18. (×630.)

Development of Puccinia graminis Secalis *in rye*

In rye numerous appressoria were seen over the stomata twenty-four hours after inoculation, and although few entries had been made, they exceeded in number those found in couch-grass at the same time. The guard cells of the stomata of entry were usually dead and discoloured as in couch-grass. Approximately 5 per cent. of the appressoria had produced vesicles in rye by the end of the first day, but no infecting hyphae had been formed.

The fungus advanced more rapidly in rye than in couch-grass. By the third day long infecting hyphae had developed, and many of these had produced haustoria. The infecting hyphae always took a course parallel to the leaf surface, and the first haustorium was formed in the epidermal cell adjacent to the stoma of entry.

By the fifth day an intercellular mycelium was well developed, and haustoria were numerous in both mesophyll and epidermal cells. Leaf areas fixed at this time showed that vesicles were still being formed in the substomatal cavities.

On the seventh day numerous pustules were found on both surfaces

of the leaves. The mycelium was dense in the mesophyll tissue underlying the pustules.

As in couch-grass, the relation between host and rust was congenial.

Development of Puccinia graminis Secalis *in barley*

The early development of the rust in barley was similar to that in rye. Appressoria were abundant by the end of the first day, but the fungus had rarely entered the leaves. The fungus had already shrivelled in one or two of the entries.

New vesicles were still being formed on the third day. No haustoria were seen in barley until the fifth day, and they were comparatively rare. As always in this rust, the first haustorium was formed in an epidermal cell adjacent to the stoma of entry. Haustoria were smaller than those in couch-grass and rye.

Leaf areas fixed seven and ten days after inoculation still showed no trace of an intercellular mycelium. Appressoria were still seen over the stomata, and occasionally empty vesicles were found in the substomatal cavities. The guard cells of the entered stomata were invariably dead and discoloured. It was apparent that barley was not a favourable host for *P. graminis Secalis* under the conditions of the experiment.

No material was fixed after the tenth day. In this particular experiment (No. 4, Table VI), a few minute pustules appeared on some leaves twenty-one days after inoculation. It is evident therefore that some entries resulted in the establishment of an intercellular mycelium, but since no material was fixed nothing can be said concerning the host-parasite relation in these infections which produced minute pustules.

Development of Puccinia graminis Secalis *in wheat*

In Wilhelmina wheat, only one small vesicle was seen in the leaf areas fixed twenty-four hours after inoculation, although many appressoria were seen over the stomata. The guard cells were always dead.

The rust made little progress during the next two days, and entries were rare. On the fifth day vesicles were more common, but they were usually empty. Some had given rise to infecting hyphae but these were poorly developed and none had formed haustoria.

The seven- and ten-day fixations showed no further development of the fungus. Not a single haustorium was found in any of the leaf areas.

Apart from the dead guard cells where the fungus was seen, there was no damage to the leaf tissues, the fungus making no progress after entry.

The development of the rust in Norka wheat was similar to that in Wilhelmina wheat. A few entries were seen, but no haustoria were formed.

In this particular experiment (No. 4, Table VI) neither variety of wheat became infected, but it should be pointed out that in one of the other experiments (No. 6, Table VI) a weak infection was obtained on Wilhelmina wheat.

Development of Puccinia graminis Secalis *in oats*

Appressoria were formed over the stomata, but few entries were made in oats. The guard cells of stomata bearing appressoria were always dead, being discoloured whether the fungus entered or not.

A few vesicles were found twenty-four hours after inoculation, but no infecting hyphae had developed. Newly formed vesicles were seen in the later fixations, occasionally even as late as the tenth day, but neither infecting hyphae nor haustoria were seen in oats.

Apart from the killed guard cells there was no damage to the leaf tissues in oats.

Discussion of experiments with *Puccinia graminis Secalis*

As was pointed out earlier, macroscopic observations of the experiments with *P. graminis Secalis* indicate that only rye and couch-grass, amongst the plants inoculated, were susceptible to this rust. Two plants of barley, out of a total of sixty-five inoculated, produced pustules, and these were minute and late in developing. Similarly, in Wilhelmina wheat, a weak infection resulted on one plant, out of a total of seventy-three inoculated. Norka wheat and oats were consistently immune.

Microscopic study of fixed leaf areas from Exp. 4 showed that the fungus entered all these plants.

Rye and couch-grass were the only true hosts for the rust. From the beginning the fungus developed more rapidly in rye, and pustules were formed three days earlier than in couch-grass.

In barley infecting hyphae occasionally produced haustoria in the epidermal cells adjacent to the stomata of entry, but no further progress was made. In wheat no further development beyond the formation of infecting hyphae was made, while in oats no infecting hyphae developed from the vesicles.

In all the plants inoculated it was found that, with very few exceptions, the guard cells of the stomata bearing appressoria were killed, whether or not entry was made. Sometimes, in entries in rye and couch-grass, it seemed that only part of the guard cell was injured, and it is perhaps significant that, in all such cases, the discoloured and shrunken end of the guard cell was found always to be the end

which was in contact with the epidermal cell in which the first haustorium was produced.

Finally, mention should be made of the interesting peculiarity that in *P. graminis Secalis* the first haustorium is always formed in an epidermal cell adjacent to the stoma of entry.

SUMMARY

1. Inoculation experiments have been carried out with uredospores of *Puccinia triticina* Erikss., *P. glumarum Tritici* Erikss., *P. anomala* Rostrup, *P. coronata* Corda, *P. graminis Secalis* Erikss., both on their normal hosts and on cereals on which they do not normally occur ("inappropriate hosts").

2. The development of these fungi in the "inappropriate hosts" has been compared with their development in their normal hosts.

3. In general, the relation of the host to the fungus which does not develop on it in nature is one of antagonism, for the mesophyll cells near the stomata of entry are usually killed. On the other hand, the initiation of invasion by these fungi on the "wrong hosts" proceeds normally at first. Exceptionally, the fungus on the "wrong host" kills the guard cells of the stomata of entry, but, failing to develop further than the formation of substomatal vesicles, leads to no further antagonistic reaction on the part of the host.

4. *P. triticina* is not very sharply specialised to wheat, for it sometimes produces normal pustules of uredospores on rye. The different types of reaction between this rust and rye are described. Occasionally minute abortive pustules of this rust were produced on barley, but the relation is usually antagonistic. In oats progress by this rust was negligible, and only once was a haustorium seen.

5. *P. glumarum Tritici* produced intercellular mycelia in rye and barley, but no pustules were formed. Although the fungus entered oats no mycelium developed.

6. *P. anomala* initiated invasion of wheat, rye and oats, killing the guard cells of the stomata of entry but failing to form mycelia in the tissues.

7. *P. coronata* Corda (uredospores from oats) entered wheat, barley, rye, *Lolium perenne* and *L. italicum*, but usually failed to develop beyond the formation of infecting hyphae. The contiguous mesophyll cells were sometimes killed.

8. The initiation of infection by aecidiospores of *Puccinia coronata* Corda from *Rhamnus catharticus* is described on oats, wheat, rye, barley and *Lolium* spp. Progress of the fungus in these hosts was negligible (except in rye), and no uredospores were formed. It is concluded that this biologic form has a host range outside the hosts experimented with.

9. *Puccinia graminis Secalis* from rye and *Agropyron repens* entered wheat, barley and oats, and occasionally produced small pustules of uredospores on barley and wheat. No infecting hyphae were produced from the substomatal vesicles in oats. In the development of *Puccinia graminis Secalis* the first haustorium is always formed in an epidermal cell adjacent to the stoma of entry.

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REFERENCES

(1) Allen, Ruth F. "A cytological study of infection of Baart and Kanred wheats by *Puccinia graminis tritici*." *J. agric. Res.* xxiii (1923), 131.
(2) —— "Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici*, forms III and XIX." *J. agric. Res.* xxvi (1923), 571.
(3) —— "Cytological studies of Forms 9, 21 and 27 of *Puccinia graminis tritici* on Khapli emmer." *J. agric. Res.* xxxii (1926), 701.
(4) —— "A cytological study of *Puccinia triticina*, Physiologic Form II, on Little Club wheat." *J. agric. Res.* xxxiii (1926), 201.
(5) —— "A cytological study of orange leaf rust, *Puccinia triticina*, Physiologic Form II, on Malakoff wheat." *J. agric. Res.* xxxiv (1927), 697.
(6) —— "A cytological study of *Puccinia glumarum* on *Bromus marginatus* and *Triticum vulgare*." *J. agric. Res.* xxxvi (1928), 487.
(7) Eriksson, J. "Über die Specialisierung des Parasitismus bei den Getreiderostpilzen." *Ber. dtsch. bot. Ges.* xii (1894), 292.
(8) —— "Nouvelles études sur la Rouille Brune des céréales." *Ann. Sci. nat.* (8), ix (1899), 241.
(9) Gibson, C. M. "Notes on infection experiments with various Uredineae." *New Phytol.* iii (1904), 184.
(10) Hoerner, G. R. "Biologic forms of *Puccinia coronata* on oats." *Phytopathology*, ix (1919), 309.
(11) —— "Infection capabilities of Crown Rust of oats. *Phytopathology*, xii (1922), 4.
(12) Hungerford, C. N. and Owens, C. E. "Specialised varieties of *Puccinia glumarum*, and hosts for variety *tritici*." *J. agric. Res.* xxv (1923), 363.

(13) Mains, E. B. "Host specialisation of barley leaf rust, *Puccinia anomala*." *Phytopathology*, xx (1930), 873.
(14) Marryat, Dorothea C. E. "Notes on the infection and histology of two wheats immune to the attacks of *Puccinia glumarum*, yellow rust." *J. agric. Sci.* II (1908), 129.
(15) Mehta, K. C. "Observations and experiments on cereal rusts in the neighbourhood of Cambridge, with special reference to their annual recurrence." *Trans. Brit. mycol. Soc.* VIII (1923), 142.
(16) Newton, Margaret. "Studies in wheat stem rust." *Trans. roy. Soc. Can.* XVI (1922), 153.
(17) Pole-Evans, I. B. "The cereal rusts. I. The development of their uredomycelia." *Ann. Bot.* XXI (1907), 441.
(18) Ruttle, Mable L. and Fraser, W. P. "A cytological study of *Puccinia coronata* Corda, on Banner and Cowra oats." *Univ. Calif. Publ. Bot.* XIV (1927), 21.
(19) Stakman, E. C. "A study in cereal rusts: Physiological races." *Bull. Minn. agric. Exp. Sta.* No. 138 (1914).
(20) —— "Relation between *Puccinia graminis* and plants highly resistant to its attack." *J. agric. Res.* IV (1915), 193.
(21) Stakman, E. C. and Levine, M. N. "The determination of biologic forms of *Puccinia graminis* on *Triticum* spp." *Tech. Bull. Minn. agric. Exp. Sta.* No. 8 (1922).
(22) Stakman, E. C. and Piemeisel, F. J. "A new strain of *Puccinia graminis*." (Abstract.) *Phytopathology*, VII (1917), 73.
(23) —— —— "Biologic forms of *Puccinia graminis* on cereals and grasses." *J. agric. Res.* X (1917), 429.
(24) Ward, H. Marshall. "The bromes and their rust fungus, *Puccinia dispersa*." *Ann. Bot.* XV (1901), 560.
(25) —— "On the relations between host and parasite in the bromes and their brown rust. *Puccinia dispersa* (Erikss.)." *Ann. Bot.* XVI (1902), 233.

AN INVESTIGATION OF THE TEMPERATURES LETHAL TO SOME WOOD-DECAYING FUNGI

By H. B. S. MONTGOMERY, B.A.

Introduction

Timber which is apparently sound and serviceable may contain traces of infection which become obvious only when the timber is incorporated in structures and decay has advanced considerably. It is desirable to heat timber suspected of being thus infected in order to kill any fungi present. It sometimes happens that valuable ornamental carved timber becomes infected with a wood-decaying fungus, and the question arises whether it is possible to kill the fungus without damaging the wood. There is thus a need for accurate data concerning the time required at certain temperatures to kill various wood-decaying fungi under known conditions (age, moisture content, etc.).

The literature on the subject is very limited. Snell (3) exposed wood in various stages of decay to heat in sealed Mason jars. He used both dry and moist wood, and showed that a fungus in dry wood resists heat treatment much better than a fungus in moist wood. He worked with rather long time periods, combined with fairly low temperatures. Hubert (1) subjected blocks of various sizes of naturally infected timber to heat for different time periods, chiefly in a Tiemann dry kiln, to determine whether commercial kiln processes were efficient in killing wood-decaying and sap-staining fungi in timber. His time intervals were wide and his results show no great differences in resistance among the various wood-decaying fungi tested. Liese (5) experimented with agar cultures of the fungus and short periods of time at fairly high temperatures.

The following experiments were undertaken to find out what relation, if any, exists between results obtained with agar cultures and those with infected wood blocks, and also to obtain some preliminary figures for the lowest temperatures which would be lethal to various wood-decaying fungi in a reasonably short time.

Cultures of a number of common wood-decaying fungi were obtained from the Forest Products Research Laboratory, Princes Risborough, and subcultured on 2 per cent. malt agar. The fungi used were *Merulius lacrymans* (Wulf.) Fr., *Poria vaporaria* (Pers.) Fr., *Pholiota adiposa* Fr., *Lentinus lepideus* Fr., *Lenzites abietina* Bull., *Lenzites saepiaria* (Wulf.) Fr., *L. striata* Swartz, *L. trabea* Pers., *Polystictus versicolor* (Linn.)

Fr., *Polyporus hispidus* (Bull.) Fr., *Schizophyllum commune* Fr., and *Fomes fraxineus* (Bull.) Fr.

Experimental methods

For the purpose of these tests, the fungi were cultured in small blocks 3 × 1 × 1 in. of ash or Scots' pine wood as follows:

Ash	Scots' Pine
Lenzites striata	*Merulius lacrymans*
Lenzites trabea	*Poria vaporaria*
Polystictus versicolor	*Pholiota adiposa*
Polyporus hispidus	*Lenzites abietina*
Schizophyllum commune	*Lenzites saepiaria*
Fomes fraxineus	*Lentinus lepideus*

After sterilisation by autoclaving, these blocks were inoculated by placing eight to ten of them on an active malt agar culture of each fungus in a suitable glass vessel. These blocks were allowed to decay for nine months before use, so that the fungus had thoroughly permeated the blocks.

The fungi were also cultured in $\frac{3}{4}$ in. test-tubes containing 10 c.c. (approx.) of 2 per cent. malt agar as slants, and the heat treatments were carried out on these cultures when they were one month old. The treatment was applied by immersing a number of these test-tube cultures in a large bath of water maintained at the desired temperature in a thermostatically controlled oven. At each time interval, one tube culture was removed and allowed to cool. Ten pieces of the culture were then removed from the centre of the slant and transplanted on to a plate of 2 per cent. malt agar. The plates were incubated at a temperature suitable to the fungus (generally 20° C.) for at least one month, and a note was made of any growth which occurred. Where no growth occurred from any of the inocula in a plate, it was concluded that the heat treatment had killed the fungus. The results of these tests are presented in Table I.

It is most important that the inocula should be taken from a standard position in the slant. Inocula derived from the upper end of the slant proved more resistant to heat than inocula from the centre. This resistance seems to be due, in part, to the less moist conditions prevailing at the upper end.

Using these preliminary data as a guide, tests were made with the infected wood material. The 3 × 1 × 1 in. blocks were cut into 1 in. cubes, and all the cubes of wood infected with any one fungus were stored in a glass dish and kept moist. By this means it was hoped to equalise the moisture contents of the blocks for each fungus. The blocks were kept in this manner for periods of over three weeks and up to two months. This proved very satisfactory from the point of view of moisture content, but it permitted moulds to grow on the surface of the blocks. These were troublesome only on wood infected

Table I. *Results with fungi in agar*

	40° C.				55° C.				60° C.		
	15 min.	30 min.	45 min.	60 min.	15 min.	30 min.	60 min.	120 min.	15 min.	30 min.	60 min.
Merulius lacrymans	.	.	.	.	.	.	.	.	.	.	—
	.	.	.	.	.	.	.	.	.	.	—
	.	.	.	.	.	.	.	.	—	—	.
	.	.	.	.	—	—	—	—	.	.	.
	—	—	—	—	.	.	.	.	.	.	.
Poria vaporaria	.	.	.	.	.	.	.	.	.	.	—
	.	.	.	.	.	.	.	.	—	—	—
	.	.	.	.	.	.	.	.	—	—	—
	.	.	.	.	—	—	—	—	.	.	.
Lenzites abietina	.	.	.	.	.	.	.	.	.	.	—
	.	.	.	.	.	.	.	.	.	—	—
	.	.	.	.	.	.	.	.	—	—	—
	.	.	.	.	.	.	.	.	—	—	—
	.	.	.	.	—	—	—	—	.	.	.
Pholiota adiposa	.	.	.	.	.	.	.	.	.	—	—
	.	.	.	.	.	.	.	.	—	—	—
	.	.	.	.	—	—	—	—	.	.	.
Polyporus hispidus	.	.	.	.	.	.	.	.	.	—	—
	.	.	.	.	.	.	.	.	—	—	—
	.	.	.	.	—	—	—	—	.	.	.
	+	+	+	+	.	.	.	.	.	.	.
Polystictus versicolor	.	.	.	.	.	.	.	.	.	.	—
	.	.	.	.	.	.	.	.	.	—	—
	.	.	.	.	.	.	.	.	.	—	—
	.	.	.	.	+	+	—	—	—	—	—
	+	+	+	+	.	.	.	.	.	.	.

	60° C.												
	30 min.	45 min.	60 min.	75 min.	90 min.	120 min.	150 min.	180 min.	240 min.	300 min.	360 min.	420 min.	480 min.	540 min.
Lenzites trabea	.	.	+	.	.	—	.	—	—	.	.	.	.	.
	.	.	+	.	+	+	—	.	.	.	.	.	.	.
	+	.	+	+	.	—	—	—	—	.	.	.	.	.
	.	.	+	.	.	—	—	—	.	.	.	.	.	.
L. saepiaria	.	.	—	.	.	—	.	—	—	.	.	.	.	.
	+	+	+	+	.	.	.	.	.	.	.	.	.	.
	+	+	+	—	.	.	.	.	.	.	.	.	.	.
	.	—	—	—	—	.	.	.	.	.	.	.	.	.
	.	+	+	+	+	.	.	.	.	.	.	.	.	.
Lentinus lepideus	.	.	+	.	.	+	.	+	+	.	.	.	.	.
	+	+	+	+	.	.	.	.	.	.	.	.	.	.
	.	.	.	.	.	+	.	.	+	.	+	.	+	.
	.	.	.	.	.	.	.	.	.	+	+	+	+	+
Schizophyllum commune	.	.	+	.	.	+	.	+	+	.	.	.	.	.
	.	.	.	.	.	.	.	.	.	.	+	+	+	.
	.	.	.	.	.	.	.	.	.	+	+	+	.	.
	.	.	.	.	.	.	.	.	.	+	+	+	.	.
	.	.	.	.	.	.	.	.	.	—	—	—	—	—
Lenzites striata	.	.	—	.	.	—	.	.	.	.	.	.	.	.
	+	+	+	+	.	.	.	.	.	.	.	.	.	.
	+	.	+	.	+	+	—	.	.	.	.	.	.	.
	.	.	.	.	—	—	—	—	.	.	.	.	.	.
	.	.	.	.	+	+	+	+	.	.	.	.	.	.
Fomes fraxineus	.	.	+	+	.	+	+	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.	.	—	—	—	—	.
	.	.	.	.	.	—	.	—	—	—	—	—	—	.
	.	.	.	.	.	.	—	.	—	—	—	—	—	.
	.	.	.	.	.	.	.	.	—	—	—	—	—	.

Note. Each — or + indicates the absence or presence of fungal growth from a single test-tube culture.

with certain fungi, ash wood infected with *Polyporus hispidus* being particularly prone to mould attack.

Various methods of heating the wood blocks in a water bath, avoiding loss or gain of moisture, were considered. It was thought undesirable to put them in a glass vessel—such as a test-tube or a Mason jar—for treatment, since heating to the desired temperature would be a slow process, and further, exchange of moisture might occur between the block and the relatively large volume of air (either saturated or dry) in the glass vessel. Experiments were then made with methods of coating the block with some impervious paint or varnish, but, apart from the risk of vapours from the paint having a toxic effect, it was found almost impossible to render the block watertight. Finally, thin sheet rubber was used. This was first cemented with rubber solution into tubes about 4 in. long and wide enough to accommodate conveniently a 1 in. cube. After putting a block into a tube, the ends were tightly tied with twine, thus forming a watertight bag. By this method, a very small volume of free air was included and the block was in close contact with the source of heat. The rubber bags, each containing one block, were submerged, by means of a wire basket, in a large bath of water maintained at the desired temperature.* One block was removed at the end of each time period, and, after cooling, it was flamed on the surface and split in half. From this freshly exposed surface of each half, five small splints were removed and placed on prune agar in a Petri dish. Thus, in all, ten splints of wood were removed from the centre of each block. The inoculated dishes were stored at 20° C. (approx.), and where no growth occurred after one month, the fungus was considered killed. The figures are presented in Table II. To keep a check on the moisture content of the blocks during treatment, a control block was enclosed in a rubber bag, and submerged in the bath for the course of the treatment, and its moisture content was determined after cooling as a percentage of oven-dry weight. This moisture content was assumed to be similar to that of the other blocks similarly decayed and heat treated.

In view of the uniformity of the results, it would seem that the variation in moisture content of these blocks was not large enough to affect the resistance of the fungus to heat. It is also of interest that these moisture contents all permitted the fungi to grow freely.

* The temperature was kept constant by manipulation of a fine adjustment on the gas supply, a mechanical thermostat not being available. The temperature varied ± 1° C.

Table II. *Results with fungi in wood*

	40° C.						55° C.						Average
	15 min.	30 min.	60 min.	90 min.	120 min.	150 min.	15 min.	30 min.	45 min.	60 min.	90 min.	120 min.	% moisture
Merulius lacrymans	−	−	−	−	−	.	.	.	.	.	.	.	103
	−	−	−	−	.	.	.	.	.	.	.	.	
	−	−	−	−	.	.	.	.	.	.	.	.	
Poria vaporaria	+	+	+	+	+	.	.	.	.	.	.	.	59
	.	.	.	.	.	.	−	−	−	−	−	−	
	.	.	.	.	.	.	−	−	−	−	−	−	
	.	.	.	.	.	.	−	−	−	−	−	−	
Lenzites abietina	.	.	+	+	+	.	.	.	.	.	.	.	105
	.	.	.	.	.	.	−	−	−	−	−	−	
	.	.	.	.	.	.	−	−	−	−	−	−	
	.	.	.	.	.	.	−	−	−	−	−	−	
	.	.	.	.	.	.	−	−	−	−	−	−	
Pholiota adiposa	+	.	+	+	+	−	.	.	.	.	.	.	35
	.	.	.	.	.	.	−	−	−	−	−	−	
	.	.	.	.	.	.	−	−	−	−	−	−	
	.	.	.	.	.	.	−	−	−	−	−	−	
Polyporus hispidus	.	.	.	.	.	.	−	−	−	−	−	−	46
	.	.	.	.	.	.	−	−	−	−	−	.	
	.	.	.	.	.	.	−	−	−	−	.	.	
	.	.	.	.	.	.	−	−	−	−	−	.	
Polystictus versicolor	.	.	.	.	.	.	+	+	.	+	−	−	55
	.	.	.	.	.	.	+	+	+	−	−	−	
	.	.	.	.	.	.	+	+	+	+	−	−	

	60° C.						65° C.					Average
	15 min.	30 min.	60 min.	90 min.	120 min.	150 min.	15 min.	30 min.	60 min.	90 min.	120 min.	% moisture
Lenzites trabea	.	.	.	−	−	−	.	.	.	.	.	46
	.	−	−	−	−	−	.	.	.	.	.	
	.	+	+	−	−	−	.	.	.	.	.	
	+	+	+	+	−	.	.	.	.	.	.	
L. saepiaria	.	.	+	−	−	−	.	.	.	.	.	63
	.	+	+	+	+	−	.	.	.	.	.	
	.	+	−	−	−	−	.	.	.	.	.	
	.	+	+	−	−	−	.	.	.	.	.	
	.	+	−	−	−	−	.	.	.	.	.	
Lentinus lepideus	.	.	.	.	.	.	.	+	−	−	−	45
	.	.	.	.	.	.	.	+	−	−	−	
	.	.	.	.	.	.	.	−	−	−	−	
	.	.	.	.	.	.	+	+	−	−	−	
	.	+	+	+	−	−	.	.	.	.	.	
Schizophyllum commune	.	.	.	.	.	.	.	−	−	.	−	31
	.	.	.	.	.	.	+	−	−	.	−	
	.	.	.	.	.	.	.	−	−	−	−	
	.	.	+	+	+	.	.	.	.	.	.	
Lenzites striata	.	.	.	.	.	.	.	−	−	−	−	61
	.	.	.	.	.	.	.	−	−	−	−	
	.	.	.	.	.	.	.	−	−	.	−	
	.	.	+	−	−	.	.	.	.	.	.	
Fomes fraxineus	.	.	.	.	.	.	.	−	−	−	−	54
	.	.	.	.	.	.	.	−	−	−	−	
	.	.	.	.	.	.	.	−	−	.	−	
	.	.	−	−	−	.	.	.	.	.	.	

Note. Each − or + indicates the absence or presence of fungal growth from a single test-block.

Results

From a study of the tabulated results it will be seen that great variation in heat resistance is shown by wood-destroying fungi.

The most resistant of the fungi tested, *Lentinus lepideus*, was killed in moist wood (1 in. cubes) by treatment for sixty minutes at 65° C.

Comparison of Tables I and II shows that close correlation exists between the times taken to kill a fungus by heat in agar culture and in wood blocks.

Summary

An account is given of experiments made to determine the time, at certain temperatures, required to kill a number of wood-decaying fungi both in agar culture and also in moist wood blocks. The results are given in tabular form.

A new method is outlined for the experimental heat treatment of wood on a small scale, by the use of rubber bags.

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REFERENCES

(1) Hubert, E. E. "Effect of kiln drying, steaming and air seasoning on certain fungi in wood." *Bull. U.S. Dep. Agric.* No. 1262 (1924), pp. 20.

(2) Liese, J. "Beobachtungen über die Biologie holzerstörender Pilze." *Angew. Bot.* XIII (1931), 138–150.

(3) Snell, W. H. "The action of heat upon the mycelium of certain structural timber fungi within wood." *Amer. J. Bot.* X (1923), 399–412.

THE PARASITISM OF *BOTRYTIS CINEREA* PERS. ON *AUCUBA JAPONICA* THUNB.

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(With Plates VI and VII)

Introduction

While a number of cuttings of *Aucuba japonica* Thunb. were being kept in sterile boiling tubes as experimental material for the investigation of a blight of that shrub it was observed that one of the cuttings became affected by a rapid brown rot which, by the end of several days, had killed it completely. As further investigation showed that the disease was due to the parasitic invasion of *Botrytis cinerea* Pers., it was considered desirable that the symptoms and effects of the disease as well as the life history of the causal agent should be described in detail sufficient to render certain a pathogenicity hitherto unrecorded for this host (1, 2).

Isolation of fungus and establishment of parasitism

The specimen on which the attack was first observed (Pl. VI, fig. 1) bore an inflorescence in the cleft between the branches of the forked stem. The fact that other cuttings without flowers after being similarly tubed for several months not only showed no signs of disintegration but had, on the contrary, produced an adventitious root system near the cut end of the stem, which rested on cotton-wool in sterile water at the base of the tube, suggested that there might be a more than fortuitous connection between the presence of an inflorescence and the occurrence of decay. It was, indeed, only at the uncuticularised surface of the nectaries that infection of undamaged twigs could be effected. This is clearly shown (Pl. VI, fig. 1) by the inability of the superficial mycelium to penetrate the intact surface of the, as yet, unattacked portion of the stem below the peduncle. The necrosis had, as indicated (Pl. VI, fig. 1), spread upwards into the younger, more succulent tissues of the limbs of the fork but when a portion of this necrotic tissue was excised after surface sterilisation and transferred on the point of a sterile scalpel to the floral nectaries of another twig the disease first spread downwards into the harder tissues of the older part of the stem (Pl. VI, fig. 2). Pieces of diseased tissues removed with the usual aseptic precautions, and planted on the sterile agar surfaces of poured plates yielded, after twenty-four to forty-eight

hours' incubation at 20° C., abundant growth of pure mycelium of characteristic *Botrytis* form from which subcultures of unquestionable purity were then obtained by hyphal tip transfers. With the fungus from diseased twigs, thus isolated in pure culture, inoculations were carried out on healthy twigs freshly cut and enclosed in sterile boiling tubes. When such a twig was abraded or incised with a sterile instrument following the surface sterilisation of selected areas, and the fungus introduced by implantation on drops of solidified sterile agar placed on these sites, the presence of brown necrotic symptoms was apparent by the end of the first day. Where the mid-rib region of the lamina, the stem apex and the nodes were inoculated (Pl. VI, fig. 3) the signs of fungal invasion, as indicated externally by the appearance of a brown rot, spread out in all directions through vascular and ground tissue alike and had reached the limits shown by the third day (Pl. VI, fig. 3). On re-isolation of the fungus from twigs diseased as a result of artificial inoculation it proved to be morphologically and culturally identical with that originally isolated and subsequently used as inoculum. Control twigs similarly treated, but for the omission of the suspected pathogen, remained perfectly healthy over the protracted period of observation.

It should be mentioned at this point that, in spite of the proved virulence of the parasitism of the fungus for healthy and even vigorously growing tissues of *Aucuba*, no evidence of disease attributable to this fungus was found in nature nor was it ever isolated from the lesions of *Aucuba* plants affected by blight or other naturally occurring form of disease. From this it seems that not only is the presence of an exposed or injured surface essential to infection by the fungus but also that the determining factor in its progressive invasion is the concomitance of the moist, enclosed conditions under which the inoculation experiments were conducted.

Pathological histology

The nature of the invasion was also examined histologically by fixing, in Flemming's strong fluid, portions taken from the externally visible region of demarcation between the brown infected and green healthy tissues and cutting longitudinal sections 4 μ thick. Examination of suitably stained sections from the apical part of a stem, including the basal portions of the petioles of the apical leaves, which had been inoculated some distance below the apex, showed that the rot was due to an indiscriminate or parenchymo-vascular attack on the tissues with intracellular penetration of the hyphae. Since the material referred to was in active growth when fungal invasion took place there was little distinction between the rate or mode of progress of the fungus in the vascular tracts and cortex and

pith respectively, except that the hyphae tended to run more longitudinally in the primordial vascular strands, where the septate character of the mycelium could readily be recognised, compared with their more tortuous course in the ground parenchyma. Stained with Flemming's triple stain the tips of the leading hyphae, retaining the safranin more strongly than the older vacuolate segments of the mycelium, showed clearly the intracellular invasive powers of the parasite (Pl. VII, fig. 1). A better stain for showing the general distribution of the pathogen in the host tissues is a solution of 1 per cent. cotton blue in lactophenol. Sections stained for three days in this medium and differentiated for about half that period in the pure solvent (lactophenol) show bright blue fungal cytoplasm and unstained hyphal walls while the walls and cytoplasm of the host cells are almost colourless and the nuclei pale greenish blue (Pl. VII, fig. 2). The central hypha shown invests the less intensely stained circular nucleus at the centre of the cortical host cell; the black spots in the body of the fungus are oil globules stained by the osmic acid of the fixative.

Identification of the pathogen

In establishing the identity of the causal agent as *Botrytis cinerea* Pers. the life history of the organism was briefly worked out in pure culture. Infected tissues from the interior of a diseased plant were sown at opposite sides of a poured plate on the sterile surface of agar prepared with a decoction of *Aucuba* twigs. Three days later typical hyaline greyish white *Botrytis* mycelium had grown out from these opposing inocula and covered almost the entire available surface of the agar with spreading hyphae which showed characteristic branchings (Pl. VII, fig. 3). After a further day or so aerial hyphae arose, principally around the margins of the Petri dish and along the junction of opposing growths, and when examined microscopically were seen to consist mainly of stouter brownish conidiophores which bore at their distal ends clusters of typical *Botrytis* conidia (Pl. VII, fig. 4). On a synthetic medium of sucrose 2 per cent., KNO_3 0·2 per cent., $MgSO_4$ $7H_2O$ and KH_2PO_4 0·05 per cent. each and washed agar 3 per cent., the mycelium after rapidly covering the available agar surface becomes denser and, probably because of that, appears much whiter. A short time later (five to six days in all) it is observed that at a number of points on the agar surface the hyphae have aggregated to form round, discrete, black sclerotia on the outside of which beadlets of exuded water can be seen (Pl. VII, fig. 5). When transferred to a fresh agar surface these sclerotia immediately germinate to produce an extensive mycelial system which, when it has exhausted the immediately available nutriment or produced a staling depressor, aggregates to form a fresh crop of resistant

sclerotia. Sclerotia are also formed at the surface of *Aucuba* stems killed by the fungus and when mature they become rounded off and fall away. In the dead leaves sclerotia of a different type are found. The fungus here forms irregular patches of pseudo-hypertrophy in the mesophyll the protuberance being towards the abaxial side and its limits often being defined by the course of the larger veins. An even more rapid production of sclerotia could be obtained by inoculating young succulent autoclaved twigs of *Aucuba* with the sporulating mycelium of *Botrytis*, when large numbers of sclerotia as well as abundant conidial fructifications were soon evident at the surface of the twigs.*

Summary

Healthy twigs of *Aucuba japonica* Thunb. are actively parasitised by *Botrytis cinerea* Pers. under conditions of enclosed humidity and provided some susceptible ingress is afforded the pathogen in the form of an unprotected nectary surface or mechanical interruption of the cuticle.

The signs of the disease and its course are described and the nature of the invasion examined histologically.

In identification, the various phases in the life history of the causal organism have been briefly outlined.

Acknowledgment

The work was carried out during part of the time the author was in receipt of a grant from the Department of Scientific and Industrial Research.

REFERENCES

(1) Oudemans, C. A. J. A. *Enumeratio Systemica Fungorum* (1919–24).
(2) Saccardo, P. A. *Sylloge Fungorum* (1882–1931).

EXPLANATION OF PLATES VI AND VII

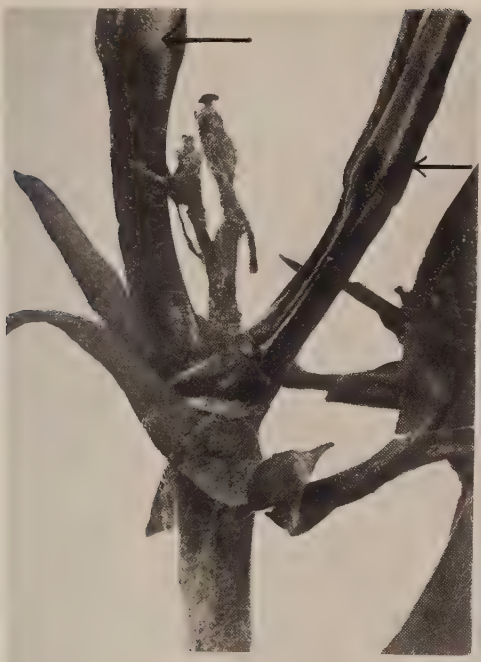
Plate VI

Fig. 1. Experimental cutting of *Aucuba* from boiling tube. *Botrytis* infection has taken place through the exposed stigmata and nectaries and the rot has spread from the peduncle into both limbs of the fork as far as the levels indicated. The growing points of the branches have been killed and the necrosis is spreading to the petioles of the apical leaves. × 2.

Fig. 2. Infection has occurred as described for fig. 1, but the disease has advanced into the older, more woody part of the stem below the peduncle to the point shown: the younger, more succulent branches, above the junction of inflorescence and main axis, have not yet been affected. × ⅔.

Fig. 3. Result, after three days, of artificial inoculations of an *Aucuba* twig with sporulating mycelium of *Botrytis* isolated from lesions similar to those depicted in figs. 1 and 2. The artificially induced lesions have spread from the sites of inoculation at (1) the mid-rib of the lower left-hand leaf at the mid-laminal region, (2) the open surface of the petiole of the left-hand apical leaf deprived of its lamina, and (3) the stem just above the lowest node shown. The limits of the general tissue rot are as defined. × 1.

* The sclerotia being produced towards the moister lower end of the twig and the conidia at the drier surface near the top.



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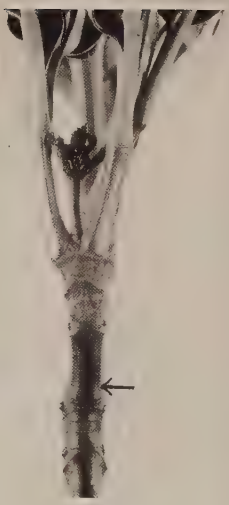

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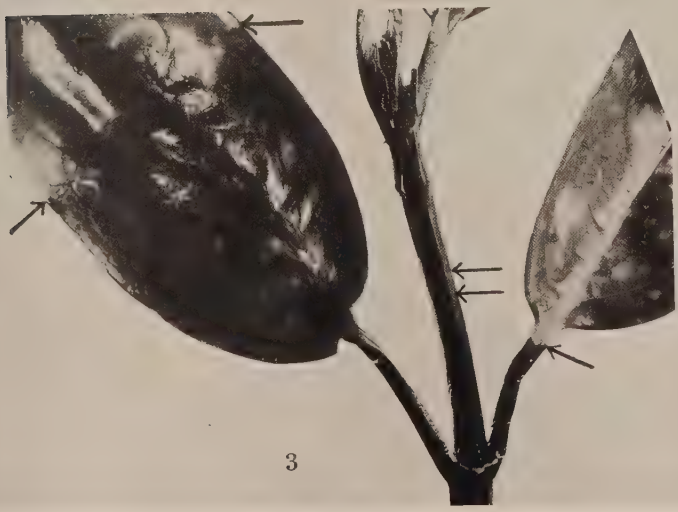

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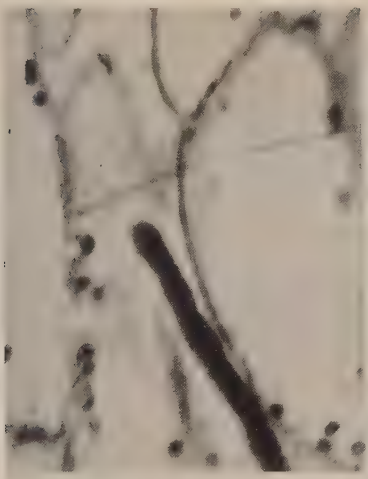

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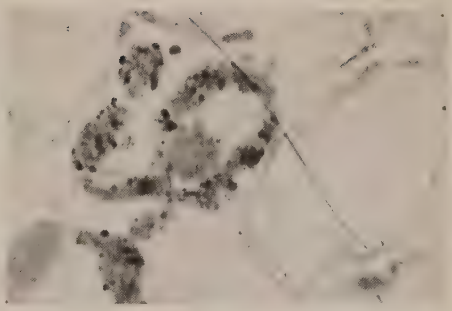

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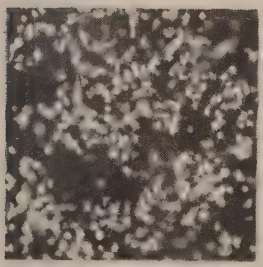

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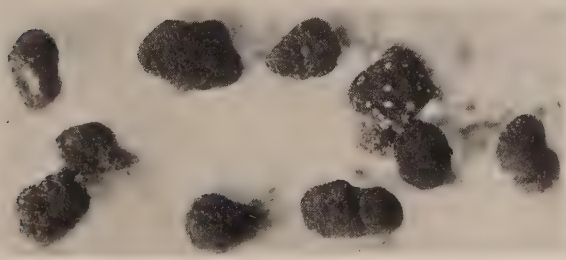

5

PLATE VII

Fig. 1. Longitudinal section of an infected *Aucuba* stem showing the parasitic *Botrytis* mycelium. The densely granular advance hypha of the invasion is penetrating the host cells of an apical petiole. Stained Flemming's triple stain. × 830.

Fig. 2. Hypha ramifying in the cells of the killed cortical region of an *Aucuba* stem (from a section similar to that described for fig. 1). The more lightly stained circular bodies are the host nuclei. Sections from material fixed in Flemming's strong fluid and stained with cotton blue in lactophenol. × 780.

Fig. 3. Re-isolation of *Botrytis* in pure culture on agar. Inocula derived from artificially infected *Aucuba* stem lesion (three days old culture). × $\frac{2}{3}$.

Fig. 4. Conidial heads of the aerial mycelium. Note typical clusters of *Botrytis* conidia. × 11.

Fig. 5. Group of *Botrytis* sclerotia standing out from dense white parent mycelium showing surface morphology and droplets of exuded moisture (from six days old subculture on nutrient agar). × 4.

Figs. 3, 4 and 5 photographed by reflected light.

NOTES ON A TECHNIQUE FOR THE LABORATORY EVALUATION OF PROTECTIVE FUNGICIDES

By R. W. MARSH

(With 1 Text-figure)

The usefulness of laboratory tests of protective fungicides has recently been pointed out by Montgomery & Moore (1) who have summarised many of the difficulties involved. Montgomery & Moore describe a method of testing based on the distribution of a drop of spray fluid of known volume over a given area on a glass slide. The writer has attempted to carry out parallel tests on slides and on leaves, and for this purpose it has been found necessary to apply the spray by means of an atomiser. The defect of glass slides is that spray fluids may spread too readily on them: this has been remedied by using slides previously covered with cellulose according to the method described by Evans & Martin (2). A brief description follows of the routine adopted in carrying out tests on slides and on leaves, together with a comparison of some of the results obtained.

Tests on slides

Glass microscope slides 3 × 1 in. are "cellulosed" by dipping them in a solution of nitro-cellulose in butyl acetate, allowing them to drain and then dry in the laboratory for two or three days. The dry cellulose film is easily detachable in water, so that, in slides that are to be leached, the film must be sealed around the glass by placing small drops of a gum such as Euparal (3) along the edge of the slide.

A standardised method of spraying the slides was adopted using an atomising apparatus of the type described by Evans & Martin (2). The essential feature of the apparatus is the two jets cut from stainless steel tubing of 0·022 in. diameter. The air pressure employed is two atmospheres and the slide is exposed for ten seconds opposite and at a distance of 2 ft. from the spray jet in the plane at right angles to the axis of the spray cone. This exposure gives an even deposit of minute droplets on the sprayed surface at the rate of 0·05 c.c. of spray fluid per square inch.

After being sprayed, the slides are allowed to dry in the laboratory for two days and then, if a leaching treatment is desired, they are submerged for an hour in a litre of fresh rainwater in a glass dish. After leaching, the slides are then again allowed to dry in the laboratory.

The spores used for a test of the toxicity of the spray deposit are conidia of *Venturia inaequalis*, taken from the youngest visible naturally occurring leaf infections on leaves of Crimson Cox apple. A suspension of these conidia is made in tap water and three drops of the suspension, each of approximately 0·015 c.c., are placed in line at 1 cm. intervals along the middle of the sprayed side of the slide. The concentration of the suspension is such that each drop contains 200–300 conidia. The inoculated slide is then inverted and enclosed over water in a Petri dish. After twenty-four hours in the laboratory a count of spore germination is made; the slide is then returned to the Petri dish and a second count is made after a further twenty-four hours.

Tests on leaves

Apple leaves of the variety Crimson Cox are employed and in the early part of the season these are obtained by cutting spurs from the tree but from July onwards extension shoots 1 ft. to 1 ft. 6 in. long are taken. A single young leaf about 1½ in. long is selected near the tip of the shoot and this leaf is allowed to remain attached to the stem, the others being removed. For the spray treatment, the procedure is exactly as described for the cellulosed slides, the stem bearing the single leaf being supported so that the leaf stands at right angles to the path of the spray with the upper surface of the leaf facing the jet. After the spray application, the stem carrying the treated leaf is placed with the cut end in water and the spray deposit is allowed to dry in the laboratory for forty-eight hours. If the leaf is to be leached it is then subjected to a sprinkling process simulating natural rainfall. In this process, distilled water is allowed to issue at the rate of twelve litres per hour from a fine rose, and the jets thus produced fall approximately a foot on to the leaf upper surface supported in a horizontal position. This treatment is continued for an hour, after which the leaf is again permitted to dry in the laboratory for a further twenty-four hours, the cut end of the stem meanwhile remaining in water.

Before inoculation, the shoot bearing the treated leaf is shortened to about 2 in. and the cut end of the shoot is placed in water in a specimen tube (3 × 1 in.). Inoculation of the leaf is carried out using the same spore suspension as is employed for the slides, two drops each of 0·015 c.c. being placed on the upper surface, one on either side of the midrib.

The inoculated leaf is immediately covered by a glass bulb (2½ in. long and 1 in. diameter) having the lower end open. This lower end is then fitted within the top of the collecting tube, water is added to fill the tube and the orifice of the bulb is thus sealed enclosing the

leaf in a moist chamber (see Fig. 1). The whole apparatus is then kept in a humid atmosphere under a bell jar in the laboratory for twenty-four hours.

At the end of this incubation period the small section of the leaf carrying one of the drops is cut out and the leaf then returned to the moist chamber as before. The excised portion is warmed on a slide in a clearing fluid made by mixing equal weights of crystalline chloral hydrate and crystalline phenol, both being first made fluid by heating. In this clearing fluid the leaf fragment becomes translucent in one or two minutes. The preparation is then examined microscopically and a count is made of the total number of spores and of the number germinated. With apple scab conidia the germ tubes are visible without staining.

Fig. 1. Apparatus for spore germination tests on leaves. (For description see text.)

After a further twenty-four hours the second inoculation drop remaining on the leaf is similarly prepared for a germination count.

It is found that preparations treated with the chloral hydrate-phenol mixture can be examined without further treatment for approximately a week after clearing.

Results

Table I sets out the results obtained with four types of treatment—no spray, lime sulphur, cuprous cyanide and cupric ferrocyanide. The lime sulphur was used at 1 per cent. strength (by volume), the copper compounds at a concentration equivalent to 2 gm. Cu per litre. The latter materials were supplied by Imperial Chemical Industries, Ltd., in the form of pastes giving excellent dispersion in water. Tests made showed that the dispersing agent used was without fungicidal action.

To avoid complexity, the results given in Table I are all taken from surfaces that have not been leached, and the table is planned to facilitate comparison between corresponding treatments on slides and on leaves. While there are certain wide variations in the germination figures relating to the same treatment, the results agree in showing that the spray deposit of cuprous cyanide in these tests was completely fungicidal on slides but of indifferent fungicidal power on leaves. Again, with the spray deposits from cupric ferrocyanide and from lime sulphur, the toxicity shown on leaf surfaces was significantly less than that shown on the cellulosed slides.

In the unsprayed series, the germination on leaves was significantly

Table I. *Percentage germinations of apple scab conidia*

	On unsprayed surfaces				On surfaces sprayed with lime sulphur				On surfaces sprayed with cuprous cyanide				On surfaces sprayed with cupric ferrocyanide			
	Germination after 24 hr.		Germination after 48 hr.		Germination after 24 hr.		Germination after 48 hr.		Germination after 24 hr.		Germination after 48 hr.		Germination after 24 hr.		Germination after 48 hr.	
1935	On slides	On leaves	On slides	On leaves	On slides	On leaves	On slides	On leaves	On slides	On leaves	On slides	On leaves	On slides	On leaves	On slides	On leaves
Apr. 26	77	**73**	.	.	11	**8**	.	**9**	0	**29**	0	.	.	.	.	.
	74	**74**	.	.	.	.	.	.	.	.	.	.	.	.	.	.
May 9	82	**84**	.	.	.	.	.	.	.	.	.	.	31	**73**	68	**92**
	70	**93**	.	.	.	.	.	.	.	.	.	.	.	.	.	.
May 16	75	**87**	75	**90**	.	.	.	.	0	**40**	0	**33**	0	**58**	0	**53**
	71	.	70	.	.	.	.	.	.	.	.	.	.	.	.	.
May 23	80	**83**	.	**91**	0	**29**	0	**90**	0	**65**	0	**25**	0	**72**	0	**70**
	72	**82**	.	**93**	.	.	.	.	.	.	.	.	.	.	.	.
May 31	.	.	80	**86**	.	.	47	**10**	.	.	.	.	.	.	0	**53**
	.	.	81	**87**	.	.	.	.	.	.	.	.	.	.	.	.
June 14	48	**83**	65	**56**	0	**0**	0	.	0	**0**	0	**0**	0	**35**	0	**60**
	66	**70**	69	**74**	.	.	.	.	.	.	.	.	.	.	.	.
June 22	79	**85**	86	**92**	0	**73**	0	**84**	0	**35**	0	**47**	.	.	.	.
	77	**87**	89	**91**	.	**78**	.	**85**	.	**51**	.	**76**	.	.	.	.
	.	.	.	.	.	**78**	.	**65**	.	**43**	.	**46**	.	.	.	.
	.	.	.	.	.	**40**	.	**46**	.	**46**	.	**24**	.	.	.	.
Mean	72	**82**	77	**84**	3	**44**	12	**56**	0	**39**	0	**36**	8	**60**	14	**66**
Standard deviation	9·0	**6·9**	8·5	**12·1**	.	.	.	.	.	.	.	.	.	.	.	.

Comparing columns 1 and 2, difference between means = 10
Standard error of above difference = 3·3
i.e. difference is significant.

Comparing columns 3 and 4, difference between means = 7
Standard error of above difference = 5
i.e. difference is not significant.

better than that on slides in the count taken twenty-four hours after sowing, but no significant difference was shown on the count taken after forty-eight hours. This would suggest a stimulating effect by the leaf sufficient to accelerate germination, but further data are required to decide this point.

Comparing the sprayed leaves with the sprayed slides, it is possible that the less effective performance of the fungicide on the former is related to a stimulatory effect of the leaf on spore germination but in considering this point the possibility of other influences must be borne in mind. If a reducing substance is produced by the leaf surface then the fungicidal effect of a sulphur-containing spray deposit might, on the hypothesis of Barker (4) be greater on a leaf than on a slide. Conversely, if the fungicidal value of a cuprous salt is dependent on its conversion to the cupric form, a reducing effect on the leaf surface should be prejudicial to the effectiveness of cuprous cyanide. It may be suggested that these biochemical influences of the leaf are outweighed by the factor of the distribution of the spray deposit. The cellulosed slide provides a perfectly plane surface while the leaf presents an epidermis diversified by irregularities, obstructed by hairs, and capable of growth after spray treatment. Therefore while the slide and the leaf may receive precisely the same spray treatment, the spray deposit encountered by the fungus spores in the germination test may be less evenly distributed on the leaf surface than on the slide. Further experiments may show whether this supposition is justified but in the meantime it appears correct to say that in the tests reported above, the living leaf has proved less flattering to the fungicide than has the slide.

Summary

1. A technique is suggested for the laboratory evaluation of protective fungicides using (*a*) cellulosed slides, (*b*) living leaves.

2. Using the same spray treatment on slides and on leaves the spray deposits from cuprous cyanide, from cupric ferrocyanide and from lime sulphur displayed less fungicidal effect on leaves than on slides.

Acknowledgments

Acknowledgments are made to my colleagues, Messrs A. C. Evans, H. G. H. Kearns and H. Martin, for inspiration and ready assistance on numerous occasions during this investigation.

The cuprous cyanide and cupric ferrocyanide preparations were provided by Imperial Chemical Industries, Ltd., who also contributed to the cost of the spraying apparatus used.

REFERENCES

(1) Montgomery, H. B. S. & Moore, M. H. "On a new method for precision testing in the laboratory of the toxicity of lime sulphur and of Bordeaux mixture as protective fungicides." *Rep. E. Malling Res. Sta.* xxii (1934), 217–22.

(2) Evans, A. C. & Martin, H. "The incorporation of direct with protective insecticides and fungicides. I. The laboratory evaluation of water-soluble wetting agents as constituents of combined washes." *Journ. Pomol. hort. Sci.* xiii (1935), 261–92.

(3) Lee, A. B. *Microtomists' Vade Mecum*, 7th edition (1913), p. 247.

(4) Barker, B. T. P. "Investigations on the fungicidal action of sulphur: third progress report." *Rep. Long Ashton Res. Sta.* (1929), pp. 130–48.

A NOTE ON THE GROWTH OF THE APPLE SCAB FUNGUS (*VENTURIA INAEQUALIS* ADERH.) ON BRAMLEY'S SEEDLING APPLES DURING THE WINTER 1934–1935

By W. F. CHEAL, D.I.C.

The breakdown in the resistance of Bramley's Seedling to attacks of the apple scab fungus has become a matter of great economic importance. In certain districts, spraying trials in 1934 showed that two pre-blossom fungicide applications in addition to the usual treatment after the flowering period were necessary in order to obtain a successful scab control: the spray programme for Bramley's equalled the drastic measures required for the most susceptible varieties, *e.g.* Worcester Pearmain.

Storage problems in 1934 were made very difficult by the large crop, and many tons of Bramley's Seedling apples were kept under outdoor conditions—in clamps, or in orchard boxes stacked and roofed with straw.

It was noticeable late in October of that year that scab colonies on Bramley's apples kept out-of-doors were unusually vigorous; fruit from spraying trials, which had been graded for scab in the previous month had to be placed in a higher category for scab infection. Even in the following month fungal growth appeared to be active, and in December it was decided to make some detailed observations.

Four Bramley's Seedling apples each about 8 oz. in weight, infected with scab, were selected on December 10, 1934, and the positions and dimensions of fifteen scab colonies were recorded. The maximum and minimum diameters of each colony were measured.

The apples were kept in a chip basket placed in a small grass enclosure at Wisbech, and the fungal colonies were measured on December 28, 1934, and again on February 13, 1935.

The figures obtained are shown in Table I.

With three exceptions, growth took place on all the colonies under observation.

The winter 1934–5 was an extremely mild one, and the high temperature for December was outstanding.

The weather conditions permitted scab to develop even on apples stored in the open. The present note therefore confirms the observations of Dr H. Wormald* who found that scab spots may increase in

* *J. Min. Agric.* September, 1934.

size on Bismarck apples in storage during winter, and this fact emphasises the importance of "pin spot" scab at picking time.

Table I

Apple	Fungus colony	Dec. 10, 1934 mm.	Dec. 28, 1934 mm.	Feb. 13, 1935 mm.
A	i	13×5	14 × 5·5	Fruit bird pecked and rotten
	ii	6×7	7 × 8	,,
B	i	7×6	7 × 6	7×6
	ii	5×4·5	6·5 × 5	7×6
	iii	5×5	5 × 5	5×5
	iv	7×6	8 × 6	8×7
	v	3×4	4 × 5	5×5
	vi	4×4	4·5 × 4	5×5
C	i	7×9	8 × 10·5	11×9
	ii	5×4	5·5 × 5	6×5·5
	iii	3×3·5	4·5 × 4	5×4·5
	iv	5×4	6 × 4·5	6×5·5
	v	7×4	7·75× 4	9×5
D	i	7×7	7 × 7	7×7
	ii	3×3	4 × 4	5×5·5

Average diameter of 13 colonies on apples B, C and D 5·076 mm. (Dec. 10, 1934), 6·23 mm. (Feb. 13, 1935).

REVIEWS

Le Genre Galera (Fr.) Quélet. By Robert Kühner. (Paul Lechevalier. Paris. Frs. 75.)

The determination of the smaller agarics is beset with difficulties, and monographs are badly needed. The well-known French mycologist Robert Kühner, has given us the results of many years' continuous work on the genus *Galera*. He describes 44 species with many varieties and forms.

The species are grouped into two genera and two sub-genera, including many of the smaller Pholiotas and Naucorias, the microscopic characters of which, in the author's view, place them among the Galeras. We regret the disappearance of *Galera* as a genus.

The descriptions are full and the microscopic characters are given in great detail. There are no coloured plates, but numerous figures of spores, basidia and cystidia. A key to the species is provided.

The author is to be heartily congratulated on his achievement.

A. A. P.

Flora Agaricina Danica. By Jakob E. Lange.

The name of Jakob E. Lange is familiar to all students of the agarics. He has produced a series of brochures that have been published at fairly regular invervals since 1914, dealing chiefly with the microscopic characters of various genera. He has always referred us to his paintings, but as they are in Denmark, few mycologists have had the advantage of consulting them. At last he has made arrangements to have these paintings reproduced under the title *Flora Agaricina Danica* and the first half of Part I has been published in Copenhagen by the Society for the Advancement of Mycology in Denmark and the Danish Botanical Society. It consists of 16 coloured plates and 40 pages of brief descriptions. Several species are on each plate, and they are splendid examples of the mycologist's art; the colourist and the systematist combining to give a recognisable picture of the species.

The figures of uncommon agarics will be of great assistance to agaricologists, and we hope that the work will receive the recognition it so fully deserves.

A. A. P.

PROCEEDINGS

Meeting held at University College, London, January 19, 1935.

President: MALCOLM WILSON, D.Sc., F.R.S.E., F.L.S.

L. E. HAWKER. Factors influencing sporulation of *Melanospora* and some other fungi.

A strain of *Melanospora destruens* was found to fruit more freely in the presence of certain other fungi than it did in pure culture. Reduction in the glucose content of the medium or transference from the normal medium to a more dilute one increased to some extent the number of perithecia formed. Media prepared from nutrient liquids in which certain other fungi had been growing were more favourable to perithecial formation than was fresh ("unstaled") medium. Evidence was obtained that the stimulatory effect of certain fungi was due to a combination of three factors, i.e. reduction in food content of the medium, production of "staling" substances by the fungus and production of a definite stimulatory substance or substances. An extract of lentils known to contain accessory substances essential for the growth of *Nematospora Gossypii* stimulated the sporulation of *Melanospora* to a striking extent. The effects of lentil extract were compared with those of certain staled media, under a variety of conditions, and it was suggested that the stimulatory substances concerned were the same or at least very similar.

R. HULL. Investigation of the control of spoilage of processed fruit by *Byssochlamys fulva*.

The presence or absence of *Byssochlamys fulva*, a spoilage agent of processed fruit, in any given material was determined by incubating at 30° C. samples of leaves, fruit and straw, collected in plugged sterile tubes filled with hot potato-sucrose agar, acidified to *p*H 3 and heated at 80° for 30 minutes, the ascospores of this fungus being resistant to this temperature.

Positive infections were obtained from diseased and healthy samples collected during several months and from several localities, including Colchester, Kent and Gloucestershire. Elimination of the fungus from the field is therefore evidently impracticable.

In studying possible control practices in the factory disinfectants were found to be ineffectual, a temperature of 92° C. was necessary to kill the ascospores in 1½ minutes, increase in sucrose up to 20 per cent. made the spores more resistant but at higher concentration the germination rate declined. It is hoped to make further investigations to discover the best way of destroying the fungus within the limits set by the canning process.

J. RAMSBOTTOM. A British species of *Kordyana*.

An account was given of the genus *Kordyana* and a new species was described which had been found on *Scirpus lacustris* in England.

Meeting held at University College, London, March 16, 1935.
President: Malcolm Wilson, D.Sc., F.R.S.E., F.L.S.

A. Burges. Mycorrhizal Investigation in Australia.

The widespread nature of fungal infection of a mycorrhizal type was emphasised and a brief account given of the extent to which mycorrhiza had been found in Australian plants. Mention was made of McLennan's work on *Lolium* and of McLuckie's on the New South Wales Orchids.

Then followed a more detailed account of the mycorrhiza of *Eriostemon Crowei* as described by McLuckie and Burges and of *Lobelia* as described by Fraser.

In *Eriostemon* two types of fungal invasion were recorded, a *Rhizoctonia* infection of the superficial cells and an extensive infection by an arbuscule- and vesicle-forming fungus. The morphological changes seen in the arbuscules and vesicles were described.

In *Lobelia* an unusual mycorrhizal infection is found. Penetration is from rhizomorphic strands and the mycelium develops extensively in the intercellular spaces which are greatly enlarged. The hyphae become filled with oil. Later the hyphae are crushed by the cells and the oil disappears; at the same time oil appears in the cells of *Lobelia*.

D. M. Cayley. Spores and spore germination in wild and cultivated mushrooms.

W. P. Findlay. Some observations on the fungi occurring in coal mines.

Fungal decay of pitwood is sometimes very rapid in shallow damp mines: this rot occurs mainly in the props used in the permanent roads and in the return airways. The majority of the pit props used in this country are of coniferous timber. The sporophores produced in mines are often abnormal; in many species of Agaricaceae the pileus is greatly reduced and the stipe is elongated and branched, while in the Polyporaceae the sterile tissue is often reduced and the fructification may consist of a pore surface only.

The principal species found attacking softwood props are *Poria Vaillantii* and other similar species of *Poria*, *Merulius lacrymans*, *Paxillus panuoides*, *Lentinus lepideus*, *Coniophora cerebella*, *Fomes annosus* and *Armillaria mellea*. On hardwood props *Polystictus versicolor* and *Stereum hirsutum* are common.

Preservation of the props by impregnation with a 2 % solution of zinc chloride or of sodium fluoride applied by the "hot and cold" open tank process, greatly increases the life of the props—pitwood treated in this way will last five to ten times as long as the untreated and, therefore, such treatment is definitely an economic proposition.

E. M. Wakefield. An edible species of *Volvaria*.

Miss Wakefield exhibited a specimen of *Volvaria volvacea*, an uncommon and edible species of a genus until recently regarded as entirely poisonous.

J. Ramsbottom and E. M. Wakefield. Mycological Nomenclature.

Mr J. Ramsbottom read a communication from Mr T. Petch which dealt with the difficulties in applying the International Rules of Botanical Nomenclature in so far as they referred to fungi. It was suggested that much of the trouble would be obviated if Saccardo's *Sylloge Fungorum* were taken as the starting point.

Mr Ramsbottom then gave a summary of the history of Botanical Nomenclature and indicated the main principles underlying the International Rules. The application of the special articles dealing with fungi was treated in detail and the ambiguities leading to different interpretations pointed out.

Miss E. M. Wakefield followed with a detailed consideration of a number of proposed *Nomina Conservanda* indicating the points at issue and how that some of the names proposed were those which would be used if the International Rules were properly applied.

LIST OF MEMBERS

Honorary Members

Bourdot, Abbé H. Saint-Priest-en-Murat per Montmarault, Allier, France. (1935.)

Lister, Miss Gulielma, F.L.S., 871, High Road, Leytonstone, Essex. (1903.) (1924.)

Rea, Mr Carleton, B.C.L., M.A., 6, Barbourne Terrace, Worcester. (1896.) (1918.)

Smith, Miss Annie Lorrain, O.B.E., F.L.S., 44, Stanwick Mansions, Stanwick Road, London, W. 14. (1899.) (1924.)

Ordinary Members

1. Aberdeen, The University Library. (1916.)
2. Adams, Rev. J. H., Landulph Rectory, Hatt, Saltash, Cornwall. (1919.)
3. Adcock, Mr Archie, Upton Road, Norwich. (1921.)
4. Ainsworth, Mr G. C., B.Sc., Experimental and Research Station, Cheshunt, Herts. (1931.)
5. Ainsworth, Mrs G. C., B.Sc., D.I.C., 3, Cromwell Avenue, Cheshunt, Herts. (1932.)
6. Alberta, University of, Edmonton, Alberta, Canada. (1924.)
7. Alcock, Mrs N. L., F.L.S., M.B.E., Royal Botanic Garden, Edinburgh. (1919.)
8. Alaily, Mr Y. A. S. El, The Botany School, Cambridge. (1935.)
9. Ashby, Mr S. F., B.Sc., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1926.)
10. Barnes, Mr B., D.Sc., Ph.D., F.L.S., Chelsea Polytechnic, London, S.W. 3. (1922.)
11. Barr, Rev. Robert, T.D., M.A., The Manse, Neilston, Renfrewshire. (1918).
12. Barrington, Dr F. J. F., University College Hospital, Medical School, University Street, London, W.C. 1. (1901.)
13. Bartlett, Mr A. W., M.A., M.Sc., F.L.S., Department of Botany, Armstrong College, Newcastle-on-Tyne. (1920.)
14. Bates, Mr G. R., c/o British South Africa Company, Mazoe Citrus Estate, Mazoe, S. Rhodesia. (1930.)
15. Bates, Mrs L. F., B.Sc., 36, Gainsborough Gardens, Golders Green, London, N.W. 11. (1921.)
16. Beardslee, Mr H. C., Perry, Ohio, U.S.A. (1933.)
17. Beaumont, Mr Albert, M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon. (1924.)

18. Bellchambers, Mr A., The Gardens, Eaton Hall, Chester. (1932.)
19. Bewley, Mr W. F., D.Sc., Experimental and Research Station, Cheshunt, Herts. (1922.)
20. Biffen, Professor Sir Rowland H., M.A., F.R.S., 136, Huntingdon Road, Cambridge. (1899.)
21. Biologist, Plant Research Laboratory, Horticultural Gardens, Burnley, Victoria, Australia. (1921.)
22. Birmingham Natural History and Philosophical Society, c/o G. T. Calvert, Esq., Hon. Librarian, Avebury House, 55, Newhall Street, Birmingham. (1920).
23. Bisby, Mr Guy R., Ph.D., Manitoba Agricultural College, Winnipeg, Canada. (1921.)
24. Blackman, Professor V. H., M.A., F.R.S., Imperial College of Science, South Kensington, London, S.W. 7. (1900.)
25. Blackwell, Miss E. M., M.Sc., Botanical Department, Royal Holloway College, Englefield Green, Surrey. (1917.)
26. Bolas, Mr B. D., M.Sc., 20, Cambridge Gardens, Winchmore Hill, London, N. 21. (1924.)
27. Bonn, Germany, Institut für Pflanzenkrankheiten, Nuss-Allee 9. (1931.)
28. Borthwick, Professor A. W., O.B.E., D.Sc., Forestry Department, The University, Aberdeen. (1911.)
29. Boston, The Mycological Club, Horticultural Hall, Boston, Mass., U.S.A. (1926.)
30. Bradshaw, Mr F., M.A., D.Sc., Armstrong College, Newcastle-on-Tyne. (1928.)
31. Braid, Professor K. W., B.A., B.Sc., West of Scotland Agricultural College, 6, Blythswood Square, Glasgow. (1922.)
32. Brazier, Mr E., Ty'n-y-gongl, Love Lane, Stourbridge. (1921.)
33. Brenchley, Mr G. H., B.A., Clare College, Cambridge. (1925.)
34. Brett, Miss M., M.Sc., Ph.D., Northern Polytechnic, Holloway Road, London, N. 7. (1921.)
35. Brierley, Professor W. B., D.Sc., F.R.A.I., F.L.S., Department of Agricultural Botany, The University, Reading. (1919.)
36. Brinton, Mr R. E. B., 68, Woodstock Avenue, Golders Green, London, N.W. 11. (1935.)
37. Brisbane, The Director, Bureau of Sugar Experiment Stations, Department of Agriculture and Stock, Queensland, Australia. (1930.)
38. British Museum, The Trustees of, Cromwell Road, South Kensington, London, S.W. 7. (1914.)
39. Brooks, Professor F. T., M.A., F.R.S., F.L.S., The Botany School, Cambridge. (1907.)

40. Brown University, Library, East Side Station, Providence, R.I., U.S.A. (1920.)
41. Brown, Professor W., M.A., D.Sc., Imperial College of Science, South Kensington, London, S.W. 7. (1922.)
42. Bruxelles, Jardin Botanique de l'État, c/o M. P. van Aerdschot. (1911.)
43. Buckley, Mr W. D., "Lynmouth", 2 Curzon Street, Slough. (1916.)
44. Buddin, Mr Walter, M.A., Laboratory of Plant Pathology, University of Reading, 7, Redlands Road, Reading. (1921.)
45. Buller, Professor A. H. R., D.Sc., Ph.D., F.R.S., University of Manitoba, Winnipeg, Canada. (1911.)
46. Bunting, Mr R. H., F.L.S., 3 Stanton Court, Weymouth. (1921).
47. Burges, Mr N. A., The Botany School, Cambridge. (1935.)
48. Burr, Mr S., M.Sc., Department of Agriculture, The University, Leeds. (1924.)
49. Butler, Mr E. J., C.I.E., C.M.G., D.Sc., M.B., F.R.S., F.L.S., Agricultural Research Council, 6 A, Dean's Yard, London, S.W. 1. (1920.)
50. Caldwell, Mr J., B.Sc., Ph.D., Department of Botany, University College, Exeter. (1932.)
51. Cambridge, The Botany School. (1920.)
52. Campbell, Mr A. H., B.Sc., Ph.D., Department of Botany, The University, Bristol. (1934.)
53. Carne, Mr W. M., F.L.S., Australia House, Strand, London, W.C. 2. (1928.)
54. Carr, Professor J. W., M.A., F.L.S., Mapperley Edge, Private Road, Sherwood, Nottingham. (1896.)
55. Carrothers, Mr. E. N., 7, Fitzwilliam Street, Belfast, N. Ireland, (1925.)
56. Carter, Miss F. M., Ph.D., Botanical Department, The University, Edgbaston, Birmingham, 15. (1934.)
57. Cartwright, Mr K. St G., M.A., F.L.S., The Red House, Kingston Blount, Oxford. (1913.)
58. Castellani, Sir Aldo, M.D., 23 Harley Street, London, W. 1. (1922.)
59. Cayley, Miss Dorothy M., John Innes Horticultural Institution, Mostyn Road, Merton Park, London, S.W. 19. (1913.)
60. Charles, Miss Vera K., United States Department of Agriculture, Bureau of Plant Industry, Washington D.C., U.S.A. (1933.)
61. Chaudhuri, Mr H., M.Sc., Ph.D., University of the Punjab, Lahore, India. (1920.)
62. Cheal, Mr W. F., Savile House, Queen's Road, Wisbech, Cambs. (1927.)

63. Chesters, Mr C. G. C., Botanical Department, The University, Edgbaston, Birmingham. (1930.)
64. Ciferri, Professor Dr R., Assistant Director, Laboratorio Crittogamico, Casella Postale 165, Pavia, Italy. (1926.)
65. Clapham, Mr A. R., M.A., Ph.D., Department of Botany, The University, Oxford. (1931.)
66. Cleland, Mr J. Burton, M.D., Professor of Pathology, University of Adelaide, South Australia. (1918.)
67. Clouston, Mr D., M.A., B.Sc. (Agr.), North of Scotland College of Agriculture, Crown Mansions, 41, Union Street (2nd Floor), Aberdeen. (1931.)
68. Colston, Miss B., B.Sc., Ph.D., The University, Manchester. (1934.)
69. Connecticut Agricultural Experiment Station, New Haven, Connecticut, U.S.A. (1929).
70. Cook, Mr W. R. I., B.Sc., Ph.D., Department of Botany, University College, Newport Road, Cardiff. (1924.)
71. Cooke, Mr G. J., 143, Newmarket Road, Norwich. (1933.)
72. Cooper, Miss Charlotte A., California Lane, Bushey Heath, Herts. (1911.)
73. Cooper, Mrs V. Astley, The Tors, Knowle, Fareham, Hants. (1921.)
74. Cornell University, The Library, New York State College of Agriculture, Ithaca, N.Y., U.S.A. (1920.)
75. Corner, Mr E. J. H., M.A., F.L.S., Assistant Director, Botanic Gardens, Singapore, Straits Settlements. (1924.)
76. Cotton, Mr Arthur D., O.B.E., F.L.S., Keeper, Herbarium, Royal Botanic Gardens, Kew, Surrey. (1902.)
77. Cunningham, Mr G. H., Ph.D., Plant Research Station, Box 442, Palmerston North, New Zealand. (1922.)
78. Curtis, Miss Kathleen M., M.A., D.Sc., D.I.C., F.L.S., Mycologist, Biological Department, Cawthron Institute of Scientific Research, Nelson, New Zealand. (1917.)
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85. Deacon, Dr G. E., Brundall, Norwich. (1933.)
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99. Ellis, Miss E. M., St Hugh's College, Oxford. (1930.)
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103. Exeter, Librarian, University College of the South-West of England. (1926.)
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108. Fisher, Mr S. D. P., Sackville Street, Leeds. (1930.)

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110. Forwood, Mr R., Minett, Muskoka, Ontario, Canada. (1930.)
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112. Gadd, Mr C. H., D.Sc., Tea Research Institute, Nuwara Eliya, Ceylon. (1921.)
113. Galloway, Mr L. D., Imperial Institute of Agricultural Research, Delhi, India. (1928.)
114. Gardner, Capt. Frederic, c/o Barclays Bank, Jersey, C.I. (1898.)
115. Garside, Mr S., M.Sc., F.L.S., Botanical Department, Bedford College, Regent's Park, London, N.W. 1. (1922.)
116. Gates, Professor R. R., D.Sc., Ph.D., F.R.S., F.L.S., King's College, Strand, London, W.C. 2. (1921.)
117. Ghamrawy, Mr Ali K., 39 Monirah Street, Cairo, Egypt. (1932.)
118. Gibson, Miss C. J., B.A., 27, Banbury Road, Oxford. (1933.)
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120. Gilbert, M. E., Docteur en Pharmacie, 6, Rue de Laos, Paris (15^{e}), France. (1924.)
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123. Glynne, Miss Mary D., M.Sc., F.L.S., Rothamsted Experimental Station, Harpenden, Herts. (1932.)
124. Gorman, Mr M. J., A.R.C.Sc.I., Albert Agricultural College, Glasnevin, Dublin. (1925.)
125. Gould, Mr F. G., Woodrising, Trapps Hill, Loughton, Essex. (1918.)
126. Green, Col. C. Theodore, A.M.S., M.R.C.S., L.R.C.P., F.L.S., 31, Shrewsbury Road, Birkenhead. (1901.)
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131. Grieve, Mr B. J., M.Sc., Botanical Department, Melbourne University, Carlton, Victoria, Australia. (1931.)
132. Grinling, Mr C. H., B.A., 71, Rectory Place, Woolwich, London, S.E. 18. (1913.)

133. Grove, Miss Jessica H., 14, The Tything, Worcester. (1927.)
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135. Hanna, Mr W. F., M.Sc., Dominion Rust Research Laboratory, Agricultural College, Winnipeg, Canada. (1925.)
136. Hansford, Mr C. G., M.A., F.L.S., Mycologist, Department of Agriculture, Kampala, Uganda. (1921.)
137. Harley, Mr J. L., Shrublands, Hethersett, Norfolk. (1932.)
138. Harris, Mr G. C. M., 148, Divinity Road, Oxford. (1934.)
139. Harris, Mr R. V., B.Sc., A.R.C.S. Horticultural Research Station, East Malling, Kent. (1924.)
140. Harrison, Mr T. H., D.Sc. Hawkesbury Agricultural College, Richmond, N.S. Wales, Australia. (1931.)
141. Harvard University, The Library, Cambridge, Mass., U.S.A. (1923.)
142. Hastings, Mr Somerville, M.S., F.R.C.S., 43, Devonshire Street, Portland Place, London, W. 1. (1913.)
143. Hawker, Miss L. E., Botanical Department, Imperial College of Science, London, S.W. 7. (1934.)
144. Heim, M. Roger, Sous-Directeur au Muséum d'Histoire Naturelle, 11, Rue de Médicis, Paris (6e), France. (1930).
145. Heimbeck, Mrs Louise, Brosoe, Levanger, Norway. (1923.)
146. Hemmi, Dr Takewo, Phytopathological Institute, Department of Agriculture, Kyoto Imperial University, Kyoto, Japan. (1923.)
147. Hereford, Mr E. H., 131, Queen Victoria Street, London, E.C. 4. (1933.)
148. Hickman, Mr C. J., Research Station, Long Ashton, nr. Bristol. (1935.)
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150. Holden, Professor H. S., D.Sc., F.L.S., Botanical Department, University College, Nottingham. (1923.)
151. Honolulu, Association of Hawaiian Pineapple Canners, P.O. Box 3166, Hawaii. (1929.)
152. Honolulu, The Library, Experimental Station, S.P.A., Box 411, Hawaii. (1920.)
153. Hopkins, Mr J. C. F., D.Sc., A.I.C.T.A., P.B. 74 B, Salisbury, S. Rhodesia. (1930.)
154. Horne, Mr A. S., D.Sc., F.L.S., F.G.S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1921.)
155. Howard, Mr H. J., F.R.M.S., F.L.S., "Lingfield", 6, College Road, Norwich. (1918.)

156. Hubbard, Miss M. D., B.Sc., Department of Botany, University College of Wales, Aberystwyth. (1933.)
157. Hughes, Mr G. C., Priory Road, Bicester. (1898.)
158. Hughes, Mr J. S., M.A., University Observatory, Oxford. (1927.)
159. Hull, The Librarian, Botanical Department, University College. (1929.)
160. Humphrey, Dr C. J., United States Department of Agriculture, Soil Conservation Service, Safford, Arizona, U.S.A. (1921.)
161. Hurrell, Mr H. E., 60, Albany Road, Great Yarmouth. (1921.)
162. Hurst, Mr C. P., Landulph Rectory, Saltash, Cornwall. (1928.)
163. Ingold, Mr C. T., M.Sc., Ph.D., Department of Botany, The University, Reading. (1935.)
164. Iowa, The Library, State University of Iowa, Library Annex, Iowa City, U.S.A. (1923.)
165. Iowa State College, Library, Ames, Iowa, U.S.A. (1927.)
166. Issatchenko, Professor B. L., Directeur du Jardin Botanique, Leningrad, Russia. (1923.)
167. John Crerar Library, 86, East Randolph Street, Chicago, Illinois, U.S.A. (1929.)
168. John Innes Horticultural Institution, Mostyn Road, Merton Park, London, S.W. 19. (1924.)
169. Johnson, Mr J. W. Haigh, M.Sc., F.I.C., F.L.S., Walton, nr Wakefield. (1919.)
170. Jones, Mr G. H., M.A., Plant Protection Section, Ministry of Agriculture, Cairo, Egypt. (1922.)
171. Jørstad, Mr Ivar, Statsmykolog, Botanisk Museum, Oslo, Norway. (1923.)
172. Keay, Miss M. A., M.A. (Cape Town), The Botany School, Cambridge. (1935.)
173. Keissler, Dr Karl, Direktor d. Botanischen Abteilung, Naturhistorisches Museum, Burgring 7, Wien 1/1, Austria. (1924.)
174. Kelly, Dr Howard A., 1418, Eutaw Place, Baltimore, Md., U.S.A. (1921.)
175. King, Miss M. E., B.A., The Botany School, Cambridge. (1935.)
176. Klika, Mr Bohumil, Hálkova 37, Prague, Vrsovice 553, Czechoslovakia. (1926.)
177. Knight, Mr H. H., M.A., The Lodge, All Saints' Villas, Cheltenham. (1914.)
178. Kuala Lumpur, F.M.S., The Director of Agriculture, Straits Settlements, and Federated Malay States. (1930.)
179. Lamb, Mr I. M., B.Sc., 18, Duke's Avenue, Kingston-on-Thames, Surrey. (1934.)

180. Lampitt, Mr L. H., D.Sc., F.I.C., Thornlea, Mount Park, Harrow, Middlesex. (1925.)
181. Leach, Mr R., B.A., Agricultural Department, Mlanje, Nyasaland. (1929.)
182. Leicester, The Museum, City of Leicester. (1923.)
183. Linder, Dr D., Farlow Herbarium, Harvard University, 20, Divinity Avenue, Cambridge, Mass., U.S.A. (1935.)
184. Line, Mr James, M.A., School of Agriculture, Cambridge. (1921.)
185. Linnean Society, The, Burlington House, Piccadilly, London, W. 1. (1919.)
186. Lloyd Library, The, 309, West Court Street, Cincinnati, Ohio, U.S.A. (1907.)
187. Loader, Miss F. M., B.Sc., Botanical Department, University College, Southampton. (1927.)
188. Lowndes, Mr A. G., M.A., F.L.S., Marlborough College, Marlborough, Wilts. (1922.)
189. Lütjeharms, Mr W. J., Assistent aan's Rijks Herbarium, Leiden, Holland. (1930.)
190. McDonald, Mr J., D.F.C., B.Sc., F.L.S., Senior Plant Pathologist, P.O. Box 338, Nairobi, Kenya Colony, East Africa. (1923.)
191. McLennan, Dr Ethel I., Botanical Department, Melbourne University, Carlton, Victoria, Australia. (1926.)
192. Madras University Library, Senate House, Triplicane, Madras, South India. (1925.)
193. Maire, M. René, D.Sc., F.M.L.S., Professeur à la Faculté des Sciences de l'Université, Algiers, Algeria, N. Africa. (1907.)
194. Marsh, Mr R. W., M.A., Research Station, Long Ashton, Bristol. (1923.)
195. Masefield, Mr G. B., c/o Department of Agriculture, Entebbe, Uganda. (1932.)
196. Mason, Mr E. W., M.A., M.Sc., F.L.S., Imperial Bureau of Mycology, 17, Kew Green, Kew, Surrey. (1921.)
197. Mason, Mrs E. W., Inglenook, 63, King's Road, Richmond, Surrey. (1922.)
198. Mason, Mr F. A., F.R.M.S., M.S.P.A., 29, Frankland Terrace, Leeds. (1912.)
199. Matthews, Professor J. R., M.A., F.L.S., Department of Botany, The University, Old Aberdeen. (1921.)
200. Mehta, Professor K. C., Ph.D., Department of Biology, Agra College, Agra, U.P., India. (1921.)
201. Melville, Mr R., B.Sc., Ph.D., 5, Courtway, Twickenham, Middlesex. (1933.)

202. Metcalfe, Mr C. R., B.A., Ph.D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey. (1926.)
203. Michigan Agricultural College Library, East Lansing, Michigan, U.S.A. (1924.)
204. Miller, Professor J. H., B.S., M.S., Ph.D., University of Georgia, Athens, Ga., U.S.A. (1930.)
205. Missouri, The Botanical Garden, St Louis, Mo., U.S.A. (1902.)
206. Mitra, Mr M., M.Sc., Ph.D., D.I.C., Assistant Mycologist, Imperial Institute of Agricultural Research, Delhi, India. (1928.)
207. Miyabe, Dr Kingo, Professor Emeritus of Botany, Hokkaido Imperial University, Sapporo, Japan. (1919.)
208. Montague, Mrs A., Penton, Crediton, N. Devon. (1898.)
209. Montreal, Canada, Faculté des Sciences, Institut Botanique, Université de Montréal. (1932.)
210. Moore, Mr W. C., M.A., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1922.)
211. Morgan, Dr G., Ashley-Hatton, Dyke Road Avenue, Brighton. (1928.)
212. Morris, Mr L. E., c/o Eton College, Windsor, Berks. (1924.)
213. Muller, Dr H. R. A., Institut voor Plantenziekten, Buitenzorg, Java. (1932.)
214. Murphy, Professor P. A., Sc.D., A.R.C.Sc.I., M.R.I.A., Department of Plant Pathology, Albert Agricultural College, Glasnevin, Dublin, N.W. 3. (1924.)
215. Murray, Mr G. H., F.E.S., Director of Agriculture, Rabaul, New Britain, Territory of New Guinea, *via* Australia. (1921.)
216. Muskett, Mr A. E., M.Sc., A.R.C.S., Queen's University, Belfast, Northern Ireland. (1923.)
217. Nannfeldt, Dr J. A., Sturegatan 11, Uppsala, Sweden. (1932.)
218. National Collection of Type Cultures, Curator, Lister Institute, Chelsea Gardens, London, S.W. 1. (1921.)
219. National Museum of Wales, Cardiff. (1924.)
220. Nattrass, Mr R. M., B.Sc. (Agric.), Ph.D., Department of Agriculture, Nicosia, Cyprus. (1925.)
221. Nebraska, The Library, University of, Lincoln, Nebr., U.S.A. (1924.)
222. Nederlandsche Mycologische Vereeniging, c/o Mr A. C. S. Schweers, Nassaulaan 17, Alkmaar, Holland. (1920.)
223. Nelson, Miss M. G., M.A., Botanical Department, The University, Oxford. (1932.)
224. New York Botanical Garden, Bronx Park, New York, U.S.A. (1904.)
225. Nicholson, Mr W. E., F.L.S., 50, St Anne's Crescent, Lewes. (1913.)

226. Noel, Miss E. F., F.L.S., 37, Burnham Court, Queen's Road, London, W. 2. (1913.)
227. North Carolina, Library, University of, Chapel Hill, North Carolina, U.S.A. (1920.)
228. Nursery and Market Garden Industries' Development Society, Ltd., Experimental and Research Station, Cheshunt, Herts. (1922.)
229. O'Connor, Mr P., Ph.D., B.Sc., A.R.C.Sc.I., National Museum, Dublin. (1925.)
230. Ogilvie, Mr L., M.A., M.Sc., Research Station, Long Ashton, nr Bristol. (1922.)
231. Oke, Mr Alfred William, B.A., F.G.S., F.L.S., 32, Denmark Road, Hove, Sussex. (1908.)
232. Ontario Agricultural College, Library, Guelph, Ontario, Canada. (1920.)
233. Oort, Dr A. J. P., Ericalaan 5, Wageningen, Holland. (1935.)
234. Osborn, Professor T. G. B., D.Sc., F.L.S., Botanical Department, The University, Sydney, N.S.W., Australia. (1910.)
235. Ottawa, Ontario, Canada, The Library, Geological Survey. (1926.)
236. Padwick, Dr G. Watts, Jealott's Hill Agricultural Research Station, Bracknell, Berks. (1936.)
237. Page, Miss W. M., M.Sc., 19, Ledam Buildings, Bourne Estate, Holborn, London, E.C. 1. (1921.)
238. Park, Mr M., Department of Agriculture, Peradeniya, Ceylon. (1929.)
239. Parke Davis & Co., Medical Research Library, P.O. Box 488, Detroit, Michigan, U.S.A. (1920.)
240. Parker, Professor C. S., Department of Botany, Howard University, Washington, D.C., U.S.A. (1932.)
241. Pearson, Mr Arthur A., F.L.S., 59, Southwark Street, London, S.E. 1. (1911.)
242. Peklo, Dr Jaroslav, Professor of Applied Botany, Bohemian Technical University, Charles Square, Prague II, Czechoslovakia. (1924.)
243. Perthshire Society of Natural Science, c/o Mr James Winter (Hon. Treasurer), 35, George Street, Perth. (1919.)
244. Petch, Mr T., B.A., B.Sc., North Wootton, King's Lynn, Norfolk. (1911.)
245. Pethybridge, Mr G. H., Ph.D., B.Sc., F.L.S., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1919.)
246. Peyronel, Dr Benjamino, R. Istituto Sup. Agrario e Forestale, Piazzale del Re, Firenze, Italy. (1932.)

247. Philadelphia, The Academy of Natural Sciences of Philadelphia, Nineteenth and The Parkway, Phil., U.S.A. (1925.)
248. Phillips, Dr H. H., 6, St John's Road, Penge, London, S.E. 10. (1923.)
249. Ping, Mr A. Wentworth, M.A., "St Olave's", Clifton, York. (1926.)
250. Potter, Rev. M. C., Sc.D., M.A., F.L.S., Corley Croft, New Milton, Hants. (1896.)
251. Preston, Mr N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop. (1920.)
252. Pretoria, South Africa, The Chief, Division of Plant Industry (91403), Department of Agriculture. (1922.)
253. Purdue University, Library, Agricultural Experiment Station, Lafayette, Ind., U.S.A. (1931.)
254. Pusa, Imperial Mycologist, Imperial Agricultural Research Institute, Delhi, India. (1921.)
255. Ramsbottom, Mr J., O.B.E., M.A., F.L.S., British Museum (Nat. Hist.), Cromwell Road, South Kensington, London, S.W. 7. (1910.)
256. Ray, Miss Anne, Penarwyn, Gorran Haven, Gorran, Cornwall. (1929.)
257. Rayner, Dr M. Cheveley (Mrs Neilson Jones), Bedford College for Women, Regent's Park, London, N.W. 1. (1921.)
258. Rea, Miss M. W., M.Sc., Salem House, Sydenham, Belfast, Northern Ireland. (1920.)
259. Rees, Mr John, M.Sc., Adviser in Agricultural Botany, University College, Cardiff. (1929.)
260. Reichert, Dr Israel, Jewish Agency for Palestine, Agricultural Experiment Station, P.O.B. 15, Rehoboth, Palestine. (1924.)
261. Rhind, Mr Donald, B.Sc., Economic Botanist, Department of Agriculture, Agricultural College, Mandalay, Burma. (1922.)
262. Rhodes, Miss Mabel, Lister Institute, Chelsea Gardens, London, S.W. 1. (1921.)
263. Robson, Mr R., M.Sc., F.Z.S., East Hanningfield, Chelmsford, Essex. (1914.)
264. Rothamsted Experimental Station, Department of Mycology, Harpenden, Herts. (1923.)
265. Rutgers College and State University of New Jersey, Library, New Brunswick, New Jersey, U.S.A. (1922.)
266. St Paul, Minnesota, U.S.A., The Library, Department of Agriculture, University Farm. (1920.)
267. Salisbury, Professor E. J., D.Sc., F.R.S., F.L.S., Botanical Department, University College, Gower Street, London, W.C. 1. (1921).

268. Salmon, Professor E. S., F.L.S., South-Eastern Agricultural College, Wye, Kent. (1922.)
269. Sampson, Miss K., M.Sc., University College, Aberystwyth, North Wales. (1920.)
270. Samuel, Mr Geoffrey, M.Sc., Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts. (1923.)
271. Scott, Mr W. W., 13, Bishop's Road, Highgate, London, N. 6. (1922.)
272. Searle, Mr G. Odell, B.Sc. (Agric.), Flax Research Institute, Flitcham Abbey, nr. King's Lynn, Norfolk. (1920.)
273. Seth, Mr N. L., B.Sc., Ph.D., D.I.C., Agricultural College, Mandalay, Burma. (1930.)
274. Sharples, Mr A., A.R.C.S., D.I.C., c/o Messrs Grindlay & Co., Parliament Street, London, S.W. 1. (1924.)
275. Shaw, Mr F. J. F., D.Sc., A.R.C.S., F.L.S., Imperial Agricultural Research Institute, Delhi, India. (1920.)
276. Shear, Dr C. L., U.S. Department of Agriculture, Bureau of Plant Industry, Washington, D.C., U.S.A. (1930.)
277. Small, Mr W., M.B.E., M.A., Ph.D., B.Sc., Director, Department of Agriculture, Zomba, Nyasaland. (1915.)
278. Smith, Mr Alexander, M.A., Ph.D., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1924.)
279. Smith, Miss K. E., The Quarry, Lutterworth Road, Nuneaton. (1913.)
280. Smith, Professor Noel J. G., Ph.D., B.Sc., Botany Department, Rhodes University College, Grahamstown, S. Africa. (1924.)
281. Smith, Mr Rupert, 38, Greenhill Gardens, Edinburgh. (1927.)
282. South London Botanical Institute, 323, Norwood Road, Tulse Hill, London, S.E. 24. (1921.)
283. Stakman, Professor E. C., University of Minnesota, Department of Agriculture, University Farm, St Paul, Minn., U.S.A. (1922.)
284. Statham, Miss E. M., 2, Westbrook Road, Blackheath, London, S.E. 3. (1926.)
285. Stationery Office, H.M., Superintendent of Publications, Book Dept., Westminster, S.W. 1. (4 subscriptions.) (1920.)
286. Stephens, Miss E. L., B.A., Department of Botany, University of Cape Town, Cape Town, South Africa. (1928.)
287. Stephens, Miss F. L., M.Sc., Department of Botany, British Museum (Natural History), Cromwell Road, South Kensington, London, S.W. 7. (1930.)

288. Steyaert, M. R. L., Station de Sélection Cotonnière de Bambesa, District des Uélés, Belgian Congo. (1931.)
289. Stirrup, Mr H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough. (1922.)
290. Storey, Mr H. H., M.A., Ph.D., East African Agricultural Research Institute, Amani, Tanganyika Territory, East Africa. (1922.)
291. Sutherland, Mr G. K., M.A., D.Sc., F.L.S., "Bremhill", 21, Combe Park, Bath, Somerset. (1914.)
292. Swanton, Mr E. W., M.B.E., A.L.S., Educational Museum, Haslemere, Surrey. (1899.)
293. Swedish Academy of Sciences, Royal, Stockholm, Sweden. (1919.)
294. Sydney, Australia, The Librarian, University of. (1922.)
295. Sydow, Herr H., Luitpoldstrasse 33, Berlin, W. 30, Germany. (1931.)
296. Tabor, Mr R. J., B.Sc., F.L.S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1914.)
297. Telfer, Mr R. Allsop, St Cuthbert's, Malvern. (1931.)
298. Tennessee, University of, Agricultural Experiment Station, Library, Knoxville, Tennessee, U.S.A. (1926.)
299. Tervet, Mr I. W., B.Sc., Edinburgh and East of Scotland College of Agriculture, 13 George Square, Edinburgh. (1933.)
300. Tetley, Miss U., Quarry Garth, Windermere, Westmorland. (1929.)
301. Tomkins, Mr R. G., M.A., Ph.D., Trinity College, Cambridge. (1925.)
302. Topping, Mrs M. P., 3, Southdown Crescent, Cheadle Hulme, Cheshire. (1930.)
303. Tunstall, Mr A. C., Tocklai Experimental Station, Cinnamara P.O., Assam, British India. (1933.)
304. Vaheeduddin Syed, Department of Plant Pathology, University Farm, St Paul, Minnesota, U.S.A. (1934.)
305. Vanterpool, Mr T. C., M.Sc., Botanical Department, University of Saskatchewan, Saskatoon, Canada. (1929.)
306. Vasudeva, Mr R. S., Cotton Pathologist, Agricultural College, Lyallpur, Punjab, India. (1929.)
307. Venkatarayan, Mr S. V., Senior Assistant Mycologist, Agricultural Department, Bangalore, S. India. (1935.)
308. Wadham, Professor S. M., M.A., Department of Agriculture, The University, Melbourne, Victoria, Australia. (1922.)
309. Wakefield, Miss E. M., M.A., F.L.S., Herbarium, Royal Botanic Gardens, Kew. (1911.)

310. Waldie, Mr J. S. L., B.Sc., C.D.A., Department of Agricultural Botany, The University, Reading. (1925.)
311. Wales, University College of, Librarian, Botanical Department, Aberystwyth, North Wales. (1927.)
312. Wallace, Mr E. R., Agricultural Institute, Kirton, nr Boston, Lincs. (1934.)
313. Wallace, Mr G. B., B.Sc. (Agric.), Ph.D., Department of Agriculture, Morogoro, Tanganyika Territory, East Africa. (1928.)
314. Wallace, Mrs G. B., B.Sc., Morogoro, Tanganyika Territory, East Africa. (1924.)
315. Wallis, Mr A., Westacre, Station Road, Kettering. (1921.)
316. Ware, Mr W. M., D.Sc., South-Eastern Agricultural College, Wye, Kent. (1924.)
317. Washington, Library, State College of, Pullman, Washington, U.S.A. (1924.)
318. Waterston, Mr J. M., B.Sc., 113, Marchmont Road, Edinburgh. (1934.)
319. Watson, Mr W., D.Sc., A.L.S., Cedene, Cheddon Road, Taunton. (1933.)
320. Westerdijk, Professor Johanna, Javalaan 4, Baarn, Holland. (1923.)
321. Western, Mr J. H., B.Sc., Department of Agricultural Botany, University College of Wales, Aberystwyth. (1934.)
322. Weston, Mr W. A. R. Dillon, M.A., School of Agriculture, Cambridge. (1923.)
323. Whetzel, Professor H. H., M.A., New York State College of Agriculture, Cornell University, Ithaca, N.Y., U.S.A. (1914.)
324. Whitaker, Mr F. Owen, 51, Grosvenor Avenue, Carshalton, Surrey. (1921.)
325. Whitehead, Mr T., D.Sc., A.R.C.S., University College of North Wales, Bangor. (1920.)
326. Wilkins, Mr W. H., M.A., D.Ph. Department of Botany, The University, Oxford. (1928.)
327. Williams, Mr P. H., 4, Belmont Villas, Windmill Lane, Cheshunt, Herts. (1930.)
328. Wilson, Miss A. P., M.B.E., A.R.C.S., 116, Fellows Road, London, N.W. 3. (1929.)
329. Wilson, Mr Alastair R., B.Sc., The Botany School, Cambridge. (1933.)
330. Wilson, Mr Malcolm, D.Sc., A.R.C.S., F.L.S., Royal Botanic Garden, Edinburgh. (1921.)
331. Wiltshire, Mr S. P., M.A., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1920.)

332. Wisconsin, The Library, University of, Madison, Wis., U.S.A. (1923.)
333. Wolf, Mr B. L., N.D.A., Cornwall Buildings, 45, Newhall Street, Birmingham. (1923.)
334. Wood, Mr F. C., 20, South Farm Road, Worthing, Sussex. (1935.)
335. Woodcock, Mr A. J. A., M.Sc., F.E.S., Rhianva, 65, Rock Avenue, Gillingham, Kent. (1926.)
336. Woodward, Mr R. C., Ph.D., Imperial Chemical Industries, Ltd., Millbank, London, S.W. 1. (1924.)
337. Woolhope, The Naturalists' Field Club, Hereford. (1896.)
338. Worcestershire Naturalists' Field Club, c/o Mr W. J. Else, Victoria Institute, Worcester. (1921.)
339. Wormald, Mr H., D.Sc., A.R.C.S., Research Station, East Malling, Kent. (1921.)
340. Yale University, Library, New Haven, Connecticut, U.S.A. (1930.)
341. Yeoman, Mr J. B., M.D., Norton, Wirral, Cheshire.
342. Young, Miss Elaine M., Ph.D., M.Sc., c/o The Forest Department, Knysna, Cape Province, South Africa. (1927.)
343. Zundel, Dr G. L. I., Botany Building, Pennsylvania State College, State College, Pa., U.S.A. (1929.)
344. Zürich, Switzerland, Botanical Garden and Museum, c/o Dr A. U. Däniker. (1921.)
345. Zürich, Institut für Spezielle Botanik der Eidg. Techn. Hochschule. (1928.)

RULES

Society's Name and Objects

1. The Society shall be called "The British Mycological Society", and its object shall be the study of Mycology in all its branches.

Members of Society

2. The Society shall consist of Honorary Members, Foundation Members, and Ordinary Members; the number of Honorary Members shall be limited to 20, and that of Foundation Members to 100, but the number of Ordinary Members shall be unlimited.

Honorary Members

3. Honorary Members shall be persons of pre-eminence in Mycology, or who have rendered special service to the Society.

Foundation Members

4. Foundation Members shall be those Members or Societies who joined the Society previous to the limit of 100 Members having been attained.*

Officers

5. The Officers of the Society shall consist of a President, one or more Vice-Presidents, Treasurer, Secretaries and Editor or Editors. They shall be elected annually, at the Annual General Meeting of the Society.

Government of Society

6. The government of the Society shall be vested in a Council, which shall consist of the President and other Officers for the time being, together with two or more other Members who shall be elected annually at the General Meeting, and one-half of whom shall retire each year and not be eligible for immediate re-election. The Members to retire shall be those who have been longest in office or, in case of equality, shall be determined by ballot. Ex-Presidents are *ex-officio* Members of the Council.

Every Meeting of the Council shall be duly summoned by the Hon. Secretary by at least seven days' notice in writing to each Member of the Council.

Period of Office

7. The Officers and Council shall hold office as from the 1st of January following their election.

* The limit of 100 Foundation Members was reached 22nd October, 1903.

Plant Pathology Committee

8. The special interests of Plant Pathology shall be delegated to an executive committee, to be called the Plant Pathology Committee of the British Mycological Society. This Committee shall consist of the President and Secretaries *ex-officio* and twelve other members of the Society. The latter shall be elected annually at the Annual General Meeting of the Society, and one-quarter shall retire in rotation each year and shall not be eligible for immediate re-election. The members to retire shall be those who have been longest in office, or, in case of equality, shall be determined by ballot.

The Officers shall consist of a Chairman and a Secretary, to be elected by the Committee each year.

At least two meetings shall be held every year, six members to form a quorum.

The Committee shall have power to appoint for any special purpose a sub-committee consisting either wholly or partly of members of the Committee.

Election of Members

9. Honorary Members shall only be elected at a Meeting of the Society by a majority of the Members then present.

All Ordinary Members shall be proposed and seconded respectively by existing Members, who shall sign a certificate (see Appendix) of recommendation, one at least of the proposers so certifying from personal knowledge. Every candidate for election shall sign an undertaking to abide by the Rules if elected (see Appendix). They shall be elected by a majority of the Members present at any meeting of the Society or by the President and Officers of the Society.

Subscription

10. All Ordinary Members and Societies shall pay an annual subscription of one pound, and Foundation Members five shillings, due on the 1st of January in each year. Honorary Members shall be exempt from any annual subscription.

Any Member wishing to retire from the Society shall give notice to the Hon. Secretary or Treasurer in writing before the 1st of December of the previous year.

Meetings

11. The Society shall hold one or more meetings annually, at a place and time determined by the Members at the preceding Annual General Meeting, or by the Council. The Annual General Meeting, for the election of officers and the transaction of other business, shall coincide with the Autumn Foray.

Accounts

12. At the Annual General Meeting of the Society in each year the Hon. Treasurer shall present duly audited accounts.

Alteration of Rules

13. The Rules shall not be altered except by a two-thirds majority of the Members present at an Annual General Meeting. A printed copy shall be sent to every Member of the Society on election, and in the event of alterations to all Members.

APPENDIX

Form of proposal for Ordinary Membership of the British Mycological Society

of.....

being desirous of becoming an Ordinary Member of the British Mycological Society, we, the undersigned Members of the Society, certify that we consider h to be a desirable Member of the Society, and beg to recommend h for election.

Dated this day of 19

....(From personal knowledge.)

Certificate to be signed by the Candidate

I hereby certify that I desire to become an Ordinary Member of the British Mycological Society and that I will abide by the Rules if elected.

.....

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